

# **Curatively intended radiotherapy for prostate cancer**

**Detection of disseminated tumor cells and long-term outcomes**

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Thesis submitted to the University of Oslo for the degree of Ph.D.  
Oslo 2009

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*Series of dissertations submitted to the  
Faculty of Medicine, University of Oslo  
No. 803*

ISBN 978-82-8072-329-1

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Cover: Inger Sandved Anfinsen.  
Printed in Norway: AiT e-dit AS, Oslo, 2009.

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## Acknowledgements

The research behind this thesis was performed from October 2004 to December 2008 when I was affiliated as a research fellow at the Faculty Division the Norwegian Radium Hospital, Faculty of Medicine, University of Oslo. The work took place at the Department of Oncology, The Norwegian Radium Hospital. In particular, I thank the included men with prostate cancer and their families for their unselfish contributions.

Professor Øyvind Bruland has been my main supervisor and mentor. He has the ability to balance between broad visions and realistic perspectives. His support has been crucial for the realization of the project. I sincerely thank him for his efforts. Dr. Wolfgang Lilleby and Professor Gunnar Kvalheim have been co supervisors. The project is partly a follow-up of Dr. Lilleby's previous Ph.D. thesis. I highly appreciate his willingness to share the great knowledge he has about prostate cancer and his many substantial contributions to the thesis. Professor Kvalheim mentored me during the writing of my first paper and was one of the main architects behind the "Micrometastases project" at the Norwegian Radium Hospital.

I'm in great debt to Professor Sophie Fosså and the "National Resource Centre for Studies of Long-term Effects after Cancer". She has actively supported me in all parts of the work and provided a stimulating working environment. Siri Hess and Vigdis Opperud offered superb administrative support. Further, I'm grateful to my additional co authors for their important contributions: Professor Jahn Nesland, Dr. Aasmund Berner, Professor Alv Dahl, Professor Trine Bjørø, Cecilie Schirmer, and Dr. Mai Aanensen.

Anne Renolen and her staff at the "micrometastases laboratory" are acknowledged for their professional processing of bone marrow samples and the unique working environment at the lab. I'm also very thankful to Dr. Bjørn Naume and Dr. Elin Borgen who willingly shared their experiences related to detection of disseminated tumor cells.

I feel lucky to have worked side by side with my colleagues and dear friends Henriette Magelssen, Kristin Reinertsen, Jan Oldenburg, Ellen Grov, Tone Skaali, Cecilie Kiserud and several other researchers at the Norwegian Radium Hospital.

I wish to thank my closest family for what they mean to me. My outstanding mother, Mette Mari, has supported me unconditionally during all phases of life. My brothers and sisters; Gulbrand, Nils Gunnar, Anne Sigrid, Jan Inge, and Helene, will always have their special places in my heart. I'm extremely proud to be the father of Astrid (14), Magnus (11), Eivind (3), and Ingvild (2). The love and affection I feel for them can not be described. Finally, I thank my beautiful and beloved Hanne for sharing her life with me.

## Abbreviations

AA	Antiandrogen
ASTRO	American Society for Therapeutic Radiology and Oncology
BM	Bone marrow
BSFI	Brief Male sexual function inventory
CT	Computed tomography
CTC	Circulating tumor cell
DTC	Disseminated tumor cell
EAU	European Association of Urology
FSH	Follicle stimulating hormone
FQ	Fatigue Questionnaire
GnRH	Gonadotropin releasing hormone
GS	Gleason score
HADS	The Hospital Anxiety and Depression Scale
HRQoL	Health-related quality of life
HT	Hormone therapy
ICC	Immunocytochemistry
LH	Luteinizing hormone
IMRT	Intensity modulated radiotherapy
ITC	Isolated tumor cell
MRI	Magnetic resonance imaging
NRH	Norwegian Radium Hospital
PCa	Prostate cancer
PCI	Prostate Cancer Index
PCR	Polymerase chain reaction
PSA	Prostate specific antigen
QLQ-C30	The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Instrument
RT-PCR	Reverse transcriptase polymerase chain reaction
RTOG	Radiation Therapy Oncology Group
SF-36	Short Form-36
SHBG	Sexual hormone binding globulin
SPCG	Scandinavian Prostate Cancer Group
TNM	Tumor–node–metastases
WHO	World Health Organization

## List of original papers

### Paper I

Berg A, Lilleby W, Bruland ØS, Fosså SD. 10-year survival and quality of life in patients with high-risk pN0 prostate cancer following definitive radiotherapy. *International Journal of Radiation Oncology\*Biology\*Physics* 2007;69:1074-1083.

### Paper II

Berg A, Dahl AA, Bruland ØS, Bjørø T, Aanensen MS, Fosså SD. Definitive radiotherapy with adjuvant long-term antiandrogen treatment for locally advanced prostate cancer. Health-related quality of life and hormonal changes. Accepted for publication in *Prostate Cancer and Prostatic Diseases*.

### Paper III

Berg A, Berner A, Lilleby W, Bruland ØS, Fosså SD, Nesland JM, Kvalheim G. Impact of disseminated tumor cells in bone marrow at diagnosis in patients with nonmetastatic prostate cancer treated by definitive radiotherapy. *Int J Cancer* 2007; 120:1603-1609.

### Paper IV

Berg A, Bruland ØS, Fosså SD, Nesland JM, Berner A, Schirmer C, Lilleby W. Disseminated tumor cells in bone marrow following definitive radiotherapy for intermediate or high-risk prostate cancer. *Prostate* 2008; 68:1607-1614.





# Chapter1: Background

## 1.1 Prostate cancer (PCa)

### 1.1.1 Epidemiology

#### *Incidence, prevalence, and mortality*

PCa is the most frequent malignancy among Norwegian men (1). In 2002–2006, the annual number of new cases was 2766 to 3829 in a population of about 4.7 million, representing 27% of all new male cancers in that period. The Norwegian incidence rates/100 000 person-years (age adjusted to the world's standard population) were 71.1 to 98.3 in the same period. PCa was responsible for 1074 deaths in 2004 (19% of all cancer deaths) and was the second-leading cause of cancer deaths in men after lung cancer. By the end of 2006, 23 389 men with a prior diagnosis of PCa were alive.

In a global perspective, the incidence of PCa has been increasing for many years, especially in industrialized countries including Norway. The incidence is particularly high in the USA and extremely low in China, giving updated age adjusted incidence rates/100 000 person-years of 124.8 and 1.7 respectively (2). The true prevalence of the disease (the number of men with diagnosed or undiagnosed PCa in a certain population at a given time) assessed by autopsy studies shows less variation than does the incidence rate (2). Because a large proportion is clinically indolent tumors, the incidence rate depends strongly on efforts to detect tumors in men without symptoms. The observed increase in incidence before the prostate-specific antigen (PSA) era has been attributed to aging of the population in general and increased attention to prostate-related symptoms combined with surgical treatment of benign hyperplasia of the prostate (3). From around 1990, when assessment of PSA in serum became common in clinical practice, a steep increase was observed in many countries (3). Different attitudes towards PSA screening of asymptomatic men may partly explain geographical differences in incidence rates (3;4). In areas with liberal screening, like the USA, a stage migration has accompanied the increased incidence rates so that a higher proportion of diagnosed tumors are organ confined than before the PSA era (5).

Mortality rates have increased constantly in many countries over the past 60 years but seem now to have reached a plateau or have declined slightly (1-4). Whether this trend can be explained by increased detection at an early stage with subsequent curative treatment is not clear (4). For unknown reasons, Scandinavian countries have the highest PCa-related mortality rates in the world (2).

### *Risk factors*

Age is the single most important risk factor. In Norway, nearly 90% of all new cases in 2006 were diagnosed in men age 60 years or older (1). A recent autopsy study of men without a history of PCa during their lifetime documented an age-dependent increase of asymptomatic tumors, with positive findings in more than 60% of men aged more than 80 years (6).

Diet is thought to play a role in both prevalence and incidence. A relatively low autopsy prevalence in Mediterranean countries (2) and higher incidence in first-generation immigrants to the USA from countries with low incidence (7) suggest that a diet rich in antioxidants based on cereals, vegetables and fish may be protective compared with a diet rich in dairy products and red meat. This view is supported by data from clinical trials (8). Specific compounds that may be chemopreventive include phytoestrogens (9), lycopenes (10), and omega-3 fatty acids (11).

Hereditary PCa is thought to explain 5–10% of all cases and is generally diagnosed six to seven years earlier than sporadic cases (12). Special interest has been directed towards inherited mutation of the tumor suppressor gene breast cancer type 2 susceptibility protein (BRCA2), which is associated with increased risk of PCa and seemingly with a particularly aggressive phenotype (13;14).

### **1.1.2 Tumor classification and staging**

In this thesis, tumors are classified using the sixth version of the tumor–node–metastases (TNM) system (Table 1.1), published in 2002 (15).

#### *T staging*

Before treatment, the local extent of the tumor is usually assessed by digital rectal examination. Transrectal ultrasound is often used as a supplement, but is not more accurate than digital rectal examination for predicting extracapsular disease (16). Neither does conventional magnetic resonance imaging (MRI) or positron emission tomography accurately predict extracapsular extension (17;18). Advances in for example MRI-based imaging techniques (19) will hopefully provide more accurate T classification because digital rectal examination underestimates extracapsular growth in about 30% of cases (16).

**Table 1.1** Clinical classification of adenocarcinoma of the prostate (15)

T1	Clinically unapparent tumor not palpable or visible by imaging
T1a	Tumor incidental histological finding in $\leq 5\%$ of resected tissue
T1b	Tumor incidental histological finding in $> 5\%$ of resected tissue
T1c	Tumor detected by needle biopsy (e.g. because of elevated PSA)
T2	Clinically apparent tumor confined within the prostate
T2a	Tumor involves $\leq$ half of one lobe
T2b	Tumor involves $>$ half of one lobe but not both lobes
T2c	Tumor involves both lobes
T3	Tumor penetrates the prostatic capsule
T3a	Extracapsular extension
T3b	Tumor invades the seminal vesicle(s)
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles; bladder neck, external sphincter, rectum, levator muscles and/or pelvic wall
N0	No regional lymph node metastases
N1	Regional lymph node metastases
Nx	Regional lymph node status not assessed
M0	No distant metastases
M1	Distant metastases

Histopathologically assessed TN classifications are referred to as pT and pN. Clinically assessed T classification is referred to as cT.

### *N staging*

The gold standard for assessing regional lymph node involvement is pelvic lymphadenectomy combined with histopathological evaluation of the lymph nodes removed. The procedure is performed in patients who are candidates for curatively intended therapy. According to the European Association of Urology (EAU) guidelines (20), patients with T-category  $\leq 2a$  and Gleason score (GS)  $\leq 6$  and PSA  $< 20\mu\text{g/L}$  may be spared lymphadenectomy because they have less than a 10% risk of lymph node involvement according to a frequently used nomogram (21). There is ongoing controversy about the optimal extent of lymph node dissection: Anatomical and clinical studies have shown that extended dissection with removal of at least 20 pelvic lymph nodes is required for optimal staging (22;23). However, two small clinical trials concluded that such extended dissection has no benefit over a more limited dissection (24;25). On the other hand, extended lymph node dissection may remove occult disease as shown in studies based on immunocytochemistry (ICC) and reverse transcriptase-polymerase chain reaction (RT-PCR) (26;27). Computed tomography (CT) and MRI are

currently not suitable for excluding lymph node involvement because of poor sensitivity (28). MRI after administration of lymphotropic superparamagnetic nanoparticles may improve the sensitivity, but this method has not been implemented in clinical practice (29).

### *M staging*

Distant metastases in PCa occur most frequently in bone followed by lung, liver, pleura, and adrenals in descending order (30). Technetium bone scan is recommended by the EAU for primary staging of bone metastases (20). Chest X-ray, ultrasound, CT, and MRI are useful if symptoms indicate soft tissue metastases. MRI may clarify inconclusive findings from the bone scan (31).

### **1.1.3 Histopathological grading**

Histopathological grading according to Gleason was recommended by a World Health Organization (WHO) consensus conference in 1993 (32) and is now the predominant grading system for adenocarcinoma of the prostate. The pattern of glandular differentiation is graded from 1 to 5, regardless of nuclear atypia. The conventional GS is the sum of the dominant and secondary grade and is noted as “dominant grade + secondary grade = GS” without reference to high-grade tertiary components (high grade refers to patterns 4 and 5). However, tertiary components of grade 4 or 5 in prostatectomy specimens add important prognostic information (33). Consequently, after a recent consensus conference, it is recommended that needle biopsy specimens with grades 3, 4 and 5 both the primary grade and the highest grade should be recorded so that tumors with conventional GS  $3 + 4 = 7$  and a tertiary grade 5 should be recorded as GS  $3 + 5 = 8$  (34). However, radical prostatectomy specimens should still be scored according to the conventional method along with a comment about the high-grade tertiary patterns. It is also recommended that a GS 2–4 in a needle biopsy specimen should not be reported because of substantial undergrading compared with the prostatectomy scores.

The “percentage of Gleason pattern 4/5” (i.e. the relative amount of the tumor that is of high grade) has been studied as an alternative or supplement to the GS (35). Further, assessment of the extent of tumor tissue in the needle biopsy specimen may give prognostic information independent of the GS (36). The tumor extent may be measured in several ways including the percentage of prostate needle biopsy cores with cancer (“percent positive biopsies”) and “percentage tumor length in all cores” (the total length of tumor tissue relative to the total length of cores).

According to a review by Humphrey of 3789 cases, the conventional GS was undergraded in needle biopsies compared with the whole gland in 42% of cases and overgraded in 15% (37). An exact correlation between needle biopsies and prostatectomy specimens was found in 43% of cases and agreement to within one score unit was found in 77% of cases. Although the GS is a robust prognostic factor, problems with inter- and intra observer variation remain to be solved (38). In clinical studies, GS is frequently categorized into three groups as  $GS < 7$ ,  $GS = 7$  or  $GS > 7$ . An alternative is dichotomization into  $GS \leq 7A$  (3 + 4) versus  $GS \geq 7B$  (4 + 3) (39).

The most common alternative grading system, called the WHO grading system, combines glandular differentiation (well differentiated, moderately differentiated, poorly differentiated or undifferentiated) and nuclear atypia (nuclear grades one to three) (40). At the Norwegian Radium Hospital (NRH), the GS replaced the WHO system as the standard grading system around 2000 (39).

In prostatectomy specimens, histopathological assessment of pT-category and extension into surgical margins and/or seminal vesicles add prognostic information that is missing in patients who are treated by radiotherapy.

#### **1.1.4 Serum biomarkers**

PSA is currently one of the most important biomarkers in oncology. The glycoprotein is normally produced in the prostate gland and acts as an enzyme to catalyze the reaction that brakes down coagulated sperm (i.e. keeps the sperm fluid after ejaculation). Several conditions are associated with elevated serum-PSA concentration, including benign hyperplasia of the prostate, prostatitis, and PCa. PSA is used routinely for diagnosis and follow-up of PCa. Additionally, bone-specific markers such as the bone isoenzym of alkaline phosphatase reflect the extent of bone metastasis (41). Several other promising markers are under investigation for use in different clinical settings (42), although none is currently in routine clinical use, and they will not be discussed further.

##### *Diagnostic PSA assessment*

Opportunistic screening (i.e. patient- or doctor-initiated PSA testing) for early detection of PCa has led to a marked increase in the incidence of PCa in Western countries. The cost has been many unnecessary biopsies and diagnoses of irrelevant tumors, which would not produce clinical symptoms during the patients' lifetime (overdiagnosis). Ongoing studies are investigating the cost–benefit of population-based screening programs (43;44). Important

problems related to PSA screening are the absence of a lower threshold that excludes clinically important cancers (Table 1.2) (45) and the low positive predictive value for positive prostate biopsies when PSA is < 10 µg/L (46).

Use of PSA isoforms to determine the free-PSA/total PSA ratio or complex PSA can probably improve the cancer-related specificity of PSA (47). PSA kinetics based on repeated PSA determinations in the lower range might help select patients with clinically relevant tumors (48).

**Table 1.2.** Prevalence of PCa diagnosed by biopsy in men with normal digital rectal examination and PSA ≤ 4 µg/L (45)

PSA	Prevalence of prostate cancer	Fraction of cancers with Gleason Score ≥ 7
≤ 0.5 µg/L	6.6%	12.5%
0.6–1.0 µg/L	10.1%	10.0%
1.1–2.0 µg/L	17.0%	11.8%
2.1–3.0 µg/L	23.9%	19.1%
3.1–4.0 µg/L	26.9%	25.0%

Abbreviations: PSA; prostate-specific antigen

### *PSA during follow-up*

PSA is used widely to monitor disease activity during follow-up of PCa patients. PSA concentration usually decreases to an undetectable or low but stable level following successful curative local therapy. Clinical relapse without a preceding increase in PSA concentration is extremely rare (49), but may occur theoretically in poorly differentiated cancers that have lost their ability to produce PSA. In contrast, increasing PSA concentration most often reflects treatment failure (50) and may allow for early secondary intervention. One problem is that increasing PSA concentration does not reliably differentiate between loco regional progression and metastatic disease. Furthermore, patients with limited life expectancy may not benefit from an intervention based on increasing PSA concentration without clinical symptoms. PSA kinetics (i.e. PSA doubling time) has been shown to add prognostic information and may help to select patients with PSA recurrence who will benefit from intervention (51).

Exact definitions of PSA recurrence following curatively intended local therapy are needed to achieve uniformity in clinical studies. The American Society for Therapeutic Radiology and Oncology (ASTRO) defined PSA recurrence following radiotherapy as three

consecutive increases in PSA concentration (the ASTRO definition) (52). A second consensus meeting in Phoenix 2005 held by ASTRO and the Radiation Therapy Oncology Group (RTOG) recommended that an increase in PSA concentration of 2 µg/L greater than the nadir value should be the standard definition of PSA recurrence following radiotherapy with or without adjuvant hormone therapy (HT) (called the Houston definition, the Phoenix definition, or the ASTRO/RTOG definition) (53). This definition has been shown to be a better predictor of subsequent clinical progression following radiotherapy than the former ASTRO definition (54). Following radical prostatectomy, two consecutive findings of PSA concentration of 0.2 µg/L or greater is a commonly used definition (55) that is recommended by the EAU (56).

Longitudinal changes in PSA concentration are also valuable to consider in a palliative setting for the response evaluation of systemic therapies such as HT or chemotherapy.

### **1.1.5 Natural history**

It is important to consider the expected prognosis in newly diagnosed PCa if left untreated when the type of treatment is to be decided. The reality of how different phenotypes behave through their life cycle will never be known completely because active treatment hampers the interpretation. Our current knowledge is based on studies of conservatively treated patients (i.e. patients without local treatment with curative intention). The following section discusses some of the studies that provided information about the natural history of PCa.

In the pre-PSA era, PCa was diagnosed mainly by of symptoms, the presence of palpable tumors, or incidental findings on specimens from transurethral resection because of prostate hyperplasia. These tumors represented a phenotypically more homogeneous group compared with T<sub>1c</sub> tumors, which are diagnosed mainly based on PSA measurements. Therefore, the natural history of localized PCa should be considered separately for tumors diagnosed before and after the introduction of PSA into routine clinical practice.

#### *Prognosis of conservatively managed localized PCa in the pre-PSA era*

Johansson et al prospectively followed 223 patients with newly diagnosed cT<sub>1-2</sub>pN<sub>x</sub>M<sub>0</sub> PCa whose treatment was delayed until progression (57). Ninety-six percent of the tumors were well or moderately differentiated. During a mean follow-up of 21 years, 36% of the tumors progressed locally and 17% of the patients developed distant metastases. Seventeen percent of the 203 patients who died during the observation period died because of PCa. The cumulative

20-year PCa-specific survival rate was 72% for patients with well-differentiated tumors and 22% for patients with moderately differentiated tumors.

Albertsen et al retrospectively studied 767 men treated by observation or immediate or delayed HT. Most of these men had cT<sub>1-2</sub> tumors, but 5% had cT<sub>3</sub> tumors and 24% had palpable tumors on digital rectal examination without further specification regarding capsule penetration. The absolute risk of dying because of PCa within a median of 24 years follow-up varied from 7% in low-grade tumors (GS 2–4) to 66% in high-grade tumors (GS 8–10).

The clinical outcomes in the observation arm of the so far only randomized study that has shown a gain in PCa-specific survival following radical prostatectomy also provided useful information about the prognosis of untreated PCa. Three hundred forty-eight men with cT<sub>1-2</sub>N<sub>x</sub>M<sub>0</sub> PCa were randomized to expectant managing and received no initial treatment except for transurethral resection of the prostate in 16% of cases (58). At 12 years follow-up, the cumulative overall mortality was 40%, the PCa-specific mortality was 18%, and the risk of distant metastases was 26%. The cumulative 10-year risk of local progression was 44% in an earlier report (59).

Although many of the patients in these studies received treatment such as transurethral resection of the prostate or HT, the results provided evidence that localized low-grade tumors rarely kill patients and that patients with high-grade tumors have a high risk of dying because of cancer within 5–10 years of diagnoses if left untreated. The outcome in men with intermediate histological differentiation shows greater variation.

#### *Prognosis of conservatively treated T<sub>1c</sub> tumors*

The outcome from conservatively treated T<sub>1c</sub> tumors will not be known until the results from two ongoing randomized trials are compiled: The Prostate Cancer Intervention versus Observation Trial (PIVOT) have randomized American men for prostatectomy or expectant management (60). The Prostate Testing for Cancer and Treatment ( ProtecT) trial randomized British PCa patients to active monitoring, definitive radiotherapy, or prostatectomy beginning in 2001 (61). Awaiting the results from these trials, Parker et al performed a modeling study to predict the natural history of PCa detected by screening (62). The results from the study by Albertsen et al (63) were adjusted for estimates of lead time (time difference between screening detection and clinical detection in the absence of screening), overdiagnosis (diagnosis of tumors that would never have been diagnosed during the patient's lifetime) (64), and overall reduction in mortality and survival benefits from early curative treatment (59). The projected 15-year cause-specific survival rates in men aged 55–59 years whose tumors



had a GS < 7, 7 and > 7 were 100%, 69%, and 29%, respectively. For men aged 70–74 years, the corresponding values were 98%, 91%, and 72%.

#### *Prognosis of conservatively managed locally advanced PCa ( $T_{3-4}N_XM_0$ )*

Tumors that are locally advanced at diagnosis have a capacity for infiltrative growth and therefore represent a subpopulation of tumors with a more uniform phenotype than localized tumors. Extracapsular growth is associated with increased risk of regional lymph node metastases as shown by Bader et al (22). Performing extensive pelvic lymph node dissection, they found lymph node metastases in 0%, 13%, 22%, 52%, and 50% of patients with pT<sub>1</sub>, pT<sub>2</sub>, pT<sub>3a</sub>, pT<sub>3b</sub>, and pT<sub>4</sub> tumors, respectively.

Patients with locally advanced tumors are usually candidates for immediate treatment, and population-based observational studies of initially untreated patients have not been performed. However, one randomized study provided useful information about the aggressiveness of this group of tumors (65). In one arm, 244 men with N<sub>X</sub>M<sub>0</sub> PCa considered too advanced for curative treatment were followed for 2.5 to 11 years. No initial treatment was given, and palliative HT was delayed until progression. The cumulative risk of distant metastases at five years was 58% (65). PCa was the cause of death in at least 60% of the patients who died within the observation period. The patients were not stratified by histopathological grading.

#### *Prognosis of conservatively managed locally advanced PCa with regional lymph node metastases ( $pN_1M_0$ )*

Schröder et al compared immediate HT versus delayed HT until progression in patients with regional lymph node metastases caused by PCa who had not received local treatment (66). The median follow-up was 8.7 years. Out of those who were randomized to delayed treatment, PCa was the cause of death in 76% who died during the observational period.

### **1.1.6 The metastatic process**

Prior to dissemination, tumor cells have to acquire the abilities to shed and migrate from the primary tumor. Molecular changes which in part may explain such phenotypical shift may be referred to as the epithelial-mesenchymal transition because they to a certain degree imitate the development of mesenchymal cells during the embryogenesis (67). Development of distant metastases frequently occurs many years after curatively intended local therapy in patients without local relapse (58;68). Therefore, dissemination of tumor cells seems to be an

early event in cancer development, and these cells may remain dormant for many years before they form overt metastases (69). This view is supported by evidence of existing single tumor cells in peripheral blood, lymph nodes, and bone marrow (BM) in many PCa patients without clinically manifest metastases (26;70-76). The tumor cells isolated from blood or BM of  $M_0$  patients seem to be genetically more heterogeneous than tumor cells isolated from patients with overt distant metastases (77;78). These findings suggest that genetic mutations, which may facilitate the formation of distant metastases, partly are acquired after the cells are shed from the primary tumor.

As early as in 1940, Batson presented evidence of metastatic spread through the pelvic veins that connect the vasculature of the spine with that of pelvic structures (79), frequently referred to as Batson's veins. He also suggested that an increase in intra-abdominal pressure facilitates venous flow from the prostate backward to the spine through these veins instead of forward to the vena cava. His findings were supported by later animal studies (80). A recent autopsy study also supported Batson's theories (30). Hematogenously disseminated PCa lesions were found in 556 men, most frequently in bone (90%), lung (46%), liver (25%), pleura (21%) and adrenals (13%) (30). Of the men with bone metastases, 90% had lesions in the spine, most frequently in the lumbar region. Involvement of the thoracic or cervical spine without lesions in the lumbar spine was rare. Furthermore, an inverse relation was found between metastases to the lung and spine. In the same study, 84% of 415 men with pelvic or para-aortic lymph node metastases from PCa also had hematogenous metastases compared with only 16% in 996 men with PCa without lymph node involvement. Thus, a biologic link between lymphatic and hematogenous dissemination seems reasonable.

The preference for disseminated PCa cells to form metastases in lymph nodes and BM may relate to several factors and is understood only partly (81). As early as in 1889, Paget introduced his "seed and soil" hypothesis (82). According to that theory, the interactions between tumor cells and the microenvironment at the distant site are crucial for whether metastases develop at that specific site. Findings from recent experimental studies support this view: Expression of chemoattractant molecules in bone and lymph nodes may direct prostate cell migration towards these organs (homing). Several possible chemoattractants with relevance to PCa have been identified (81). One is epidermal growth factor, which may direct PCa cells toward both lymph nodes and BM (83). The preferred adhesion of PCa cells to BM endothelium compared with other epithelia may facilitate the development of bone metastases (84). Furthermore, factors like insulin-like growth factor are produced by osteoblasts and stimulate the proliferation of PCa cells in vitro (85). PCa cells also affect bone homeostasis by

producing molecules that stimulate osteoblasts and osteoclasts, enhancing bone formation and bone degradation, respectively (86). Increased bone turnover may increase the release of growth factors that in turn stimulate cancer cell proliferation.

### **1.1.7 Endocrine aspects**

In 1941, Charles Higgins (1901-1997) and his research fellow Clarence Hodges (1914–2001) documented that PCa activity is inhibited when serum androgen concentration is deprived (87). Interference with androgen receptor activity has been the mainstay in systemic treatment of PCa after this finding, for which Higgins received the Nobel Prize in 1966. We now know that activation of the androgen receptor is crucial for cell proliferation and tissue maintenance both in normal prostate tissue and PCa (88). Testosterone is taken up from the blood by prostate epithelia and converted to dihydrotestosterone through an enzymatic process driven by 5 $\alpha$ -reductase. Dihydrotestosterone has strong affinity for the androgen receptor, which, subsequent to ligand binding, binds to DNA within regulatory regions of the target genes.

#### *Castration*

In men, about 95% of circulating androgens are synthesized from cholesterol in the gonadal Leydig cells (89). Nongonadal androgen production occurs mainly in the adrenals. Surgical castration leads to an immediate decrease in serum testosterone concentration and remains the gold standard in treatment of advanced symptomatic PCa. However, in clinical practice, castrate levels of testosterone are usually achieved pharmaceutically by interfering with the feedback mechanisms of the hypothalamic–pituitary–gonadal axis. The mainstay of such medical castration is currently gonadotropin releasing hormone (GnRH) agonists.

Administration of such compounds initially stimulates and, a few days later, inhibits hypothalamic production of GnRH. The subsequent decrease in the production of pituitary luteinizing hormone (LH) hampers androgen production in the testis. Endocrine side effects of castration include erectile dysfunction and loss of libido, decreased bone density, decreased muscle mass, hot flashes and possibly psychological effects such as fatigue and impaired vitality (90;91). Furthermore, long-term use of GnRH agonists is associated with increased risk of diabetes and cardiovascular disorders (92). Estradiol is synthesized from testosterone and serum estradiol concentration is low in castrated men. This is probably the reason for castration-associated loss of bone mass. Earlier, medical castration was achieved by estradiol compounds without this disadvantage. However, such treatment was associated with higher

cardiovascular mortality compared with GnRH agonists and is therefore not currently recommended standard first-line treatment (93).

Compared with radiotherapy alone for locally advanced or localized high-grade PCa, adjuvant castration-based therapy improves clinical outcomes (94-98).

### *Androgen receptor blockade*

Androgen receptor activity may be blocked without interfering directly with androgen production using antiandrogen (AA) compounds that competitively inhibit the androgen receptors. In contrast to castration-based therapy, the gonadal androgen production is not inhibited by peroral nonsteroidal AAs. Instead, testosterone and estradiol concentration increases in the serum (99) probably because of androgen receptor blockade in the pituitary gland and hypothalamus, which inhibits the negative feedback regulation that normally occurs in response to high serum sex-hormone concentrations. Breast hypertrophy and breast pain (100) are frequently occurring adverse effects, probably related to the increased estradiol concentration. Bone mineral density seems to be maintained during AA treatment (101). Preservation of sexual function is by many clinicians considered a main advantage of AAs over GnRH agonists (102). In a randomized trial, sexual and physical function was better after one year of treatment with the AA bicalutamide compared with GnRH agonist (100). However, in another study, long-term treatment with flutamide was associated with a gradual loss of erectile function over the first few years of treatment (103). The side effects from AAs and GnRH agonists have not been compared in randomized studies with longer follow-up than two years (100;101).

In locally advanced PCa, radiotherapy + AA has been shown to improve overall survival and PCA-specific survival compared with radiotherapy alone (104) and AA alone (105). AA monotherapy is not recommended for localized PCa because a randomized trial showed a trend toward poorer overall survival during such treatment compared with watchful waiting (104).

### *Castrate-resistant PCa*

In most cases, HT is only effective for a limited timeperiod. One mechanism behind tumor-progression during HT is probably related to increasing number within the tumor of neuroendocrine cells without androgen receptors (106). In addition, recent data show that androgen receptor-mediated tumor progression is important, despite castrate levels of serum androgens (107). The term “castrate-resistant PCa” is therefore now preferred over the

previously used “hormone- or androgen independent PCa”. The concentration of intracellular androgens is higher in tissues from metastatic castrate-resistant PCa than in PCa that responds to castration, suggesting that the androgens are produced by the tumor cells (autocrine activity) (108). Novel agents which inhibit the intracellular production of androgens are currently being tested (109).

The term “complete androgen blockade” refers to the combination of castration and AA. The rationale for such combined treatment is that castration does not affect the ~5% of androgen production that occurs outside the testis and that peripheral receptor blockade will improve the antitumor effect. The clinical benefits are probably limited (110). Patients with progressive PCa despite complete androgen blockade frequently experience temporary fewer symptoms and a decline in PSA concentration, a phenomenon referred to as the AA withdrawal response (111).

### **1.1.8 Curative treatment strategies**

#### *Localized tumors ( $cT_{1-2}N_0M_0$ )*

The most intriguing problem facing early detected tumors is predicting whether the individual patient will benefit from curative treatment. Given the great variation in tumor biology discussed earlier, there is a high risk of both overtreatment and undertreatment. Because of the generally slow progression of PCa, watchful waiting with symptomatic treatment at progression is reasonable in many patients with limited life expectancy. Active surveillance of patients with early detected tumors and favorable prognostic factors followed by selective curative treatment of those with evidence of progression is another approach that theoretically may help to avoid overtreatment of indolent tumors and at the same time reduces the risk of undertreating aggressive tumors (112).

If curative therapy is decided, several options are available. Radical prostatectomy is the only option shown in a randomized trial to increase PCa-specific survival compared with conservative management (58). The risk of dying from PCa was associated with positive margins in the surgical specimens. In the same study, a significant increase in overall survival was found in patients younger than 65 years. Radiotherapy, provided by external beams or internal implants (brachytherapy) or both, is generally accepted as being equally effective as prostatectomy in similar patients (113), although randomized studies have not been performed. The EAU regards cryotherapy (114) and high-intensity focused ultrasound ablation (115;116) as investigational therapies with curative potential (20).

The prognosis following curatively intended therapy for localized PCa is generally good. For patients diagnosed in the PSA-era, the 10-year mortality rate approximates zero in those with low-risk disease defined as PSA < 10 µg/L and GS < 7 and cT < 2b (117). In patients with increased risk of treatment failure related to unfavorable prognostic factors such as PSA > 10 µg/L, a GS ≥ 7 or cT<sub>2b-c</sub>, radiotherapy + castration-based HT has been shown to increase overall survival and PCa-specific survival compared with radiotherapy alone (118). A similar benefit from HT combined with prostatectomy has not been found, although adjuvant HT following prostatectomy may improve clinical outcomes in selected patients (119). Based on a relatively high risk of PCa-specific death in patients with PSA >10 µg/L and T<sub>2b-c</sub> and GS ≥ 7 following curatively intended therapy, these patients were recently suggested as candidates for trials exploring systemic treatments adjuvant to standard radical prostatectomy or radiotherapy + HT (120). Patients with cT<sub>1-2</sub> tumors that are upstaged to pT<sub>3</sub> or have tumor involvement in the resection margin following prostatectomy may benefit from adjuvant radiotherapy to the prostatic bed (121;122). Local salvage options in selected patients with PSA recurrence after primary therapy include radiotherapy following prostatectomy and prostatectomy following radiotherapy (123).

In summary, the current standard curative treatment for localized PCa is radical prostatectomy or conformal dose-escalated radiotherapy (74–78Gy). The EAU recommends adjuvant HT for poorly differentiated tumors treated by radiotherapy (20).

#### *Locally advanced tumors (cT<sub>3-4</sub>pN<sub>0</sub>M<sub>0</sub> or cT<sub>3-4</sub>pN<sub>x</sub>M<sub>0</sub>)*

Patients with locally advanced tumors at diagnosis are as previously accounted for at substantial risk of dying from PCa within 10 years if left untreated. They will usually benefit from multimodal therapy including local and systemic treatment (Boustead, BJU, 2007). Curatively intended radiotherapy + HT is the standard treatment and has been shown to improve clinical outcomes compared with radiotherapy alone in several randomized trials (94-98;104). Radiotherapy + AA has been shown to improve overall survival and PCa specific survival compared with AA alone (105). Primary prostatectomy may be an option in selected patients with small cT<sub>3a</sub> tumors (124). Primary palliative HT is reasonable in patients with short life expectancy. Lymph node-positive PCa is currently regarded as noncurable and standard treatment according to the EAU is palliative HT (20).

### **1.1.9 Advances in curatively intended radiotherapy**

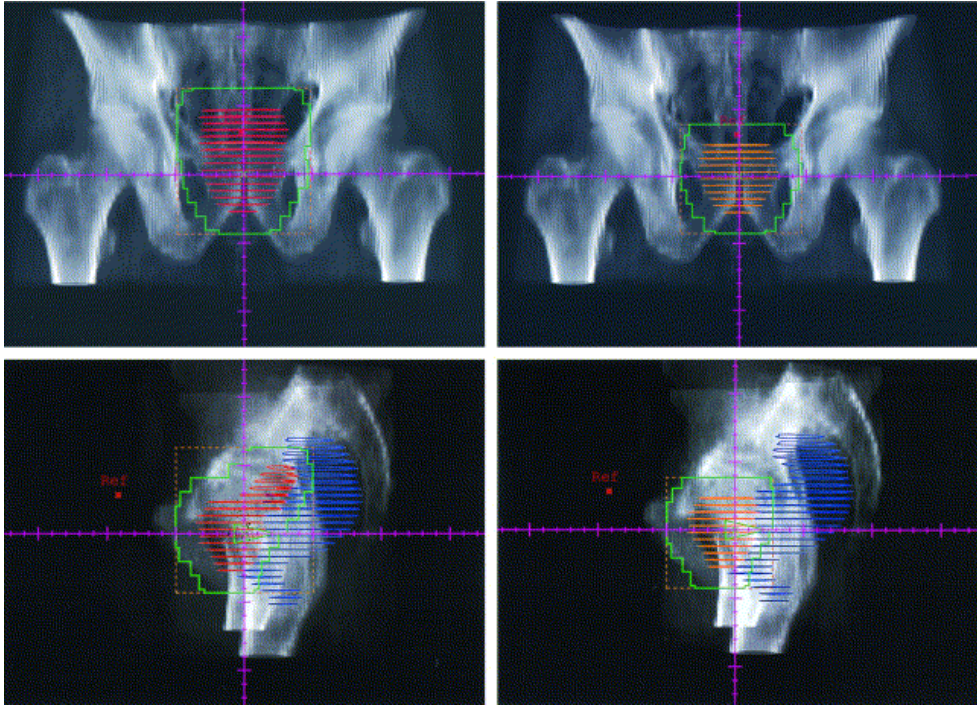
#### *Technological aspects*

The therapeutic challenge of radiotherapy for nonmetastatic PCa is to deliver an adequate dose to the tumor while avoiding toxicity to adjacent organs (i.e. rectum, bladder, urethra, and penile structures). Conventional external beam radiotherapy usually refers to two-dimensional treatment planning (125). Three-dimensional conformal external beam radiotherapy (Fig. 1.1) refers to CT-based three-dimensional planning systems that in combination with multileaf collimators in the gantry of the linear accelerators, allow for reduction of treatment volumes for normal tissues compared with conventional radiotherapy (126). Modern intensity-modulated radiotherapy (IMRT) increases the conformality further by inverse dose planning and intensity modulation of the beams (127). Brachytherapy is currently evolving as an alternative or supplement to external beam radiotherapy, and allows for conformal radiation of the prostate with a sharp decrease in dose outside the gland (125).

#### *Dose escalation*

Even though a dose–response relationship for PCa has been demonstrated (128), the optimal dose for different phenotypes of PCa has not been clarified. A previous review concluded that 70–74 Gy seems appropriate for low-risk patients and that intermediate-risk patients benefit from dose-escalation to 78–80 Gy (129). The benefit from dose escalation in high-risk patients is less obvious because of the competing risks of failure, such as undetected locoregional or distant dissemination. The role of dose escalation has not been evaluated prospectively in the setting of adjuvant HT. The authors of the review concluded that it is unclear whether high-risk patients who receive combined treatment benefit from doses > 70 Gy. Somewhat provocative to these conclusions, another study showed that a dose > 92 Gy is probably necessary to secure histologically verified eradication of locally advanced tumors (130). Abnormal postradiotherapy biopsies were reported recently in more than half of patients who received 70 or 78 Gy (131).

Well-informed patients may prefer treatment with a lower risk of toxicity to a gain in overall survival. Significantly more patients chose 70 Gy over 74 Gy when they were informed about the increased risk of rectal toxicity associated with 74 Gy, despite a survival advantage for the higher dose (132). Conformal treatment modalities such as IMRT combined with image guided radiotherapy might allow dose escalation to more than 80 Gy without unacceptable toxicity (128;133;134).



**Fig. 1.1.** Conformal radiotherapy of prostate cancer. Anteroposterior field with the vesiculae seminalis included (upper left) and without the vesiculae seminalis (upper right) in the target volume arrangement. Lateral field with the vesiculae seminalis included (lower left) and without the vesiculae seminalis (lower right) in the target volume arrangement. Reprinted from International Journal of Radiation Oncology, Biology, Physics, Vol. 43, No 4. Long-term morbidity and quality of life in patients with localized prostate cancer undergoing definitive radiotherapy or radical prostatectomy. Lilleby W, Fosså SD, Wåhre HR, Olsen DR. Page 737. Copyright (1999), with permission from Elsevier.

### *Adjuvant treatment*

In patients with locally advanced PCa or localized PCa with poor prognostic factors, both castration-based HT (94-98) and nonsteroidal AA (104) combined with radiotherapy are associated with better clinical outcomes compared with radiotherapy alone; the benefits include improved overall survival. HT may be given neoadjuvant, concomitant and/or adjuvant to radiotherapy. In addition to the possible effects of HT on occult disseminated disease, neoadjuvant and concomitant HT has important local effects. Downsizing of the prostate gland associated with HT reduces the target volume and consequently lowers the dose to the rectum (135). HT is also associated with T-cell infiltration and apoptosis of PCa cells, which may enhance the radiotoxic effects (136).



The optimal duration of HT is unclear. Two randomized trials have shown that, compared with six months of HT together with conventional radiotherapy  $\leq 70\text{Gy}$ , 28 months (137) or 36 months (138) improves PCa-specific outcomes. Results from another study suggest that treatment for more than six years may benefit some patients (139).

D'Amico et al showed recently that even though radiotherapy + six months of HT improved overall survival compared with radiotherapy alone for localized unfavorable-risk PCa, this was not true for a subgroup of patients with moderate to severe comorbidity. Future studies should focus on identifying patients for whom the disadvantages of adjuvant treatment may outweigh the antitumor effects.

#### *Radiation of pelvic lymph nodes*

Instead of performing surgical staging before radiotherapy, many centers provide a whole-pelvic radiation field ( $\sim 50\text{ Gy}$ ) to patients with a certain risk of lymph node involvement based on nomograms (21;140) followed by a boost to the prostate only. This practice is considered by many to be standard treatment after a randomized trial (RTOG 94-13) found increased progression-free survival following whole-pelvic radiotherapy + HT compared with prostate only radiotherapy + HT (141). However, a recent update of this trial showed that the difference in progression-free survival between the groups was no longer significant on prolonged follow-up (142). Preliminary results from another randomized trial (GETUG-01) did not indicate that whole-pelvic radiotherapy improves progression-free survival compared with prostate-only radiotherapy (143). Thus, the clinical benefits of whole-pelvic radiotherapy is uncertain and associated with controversies (144;145). Compared with radiation restricted to the prostate, whole-pelvic radiation is associated with increased risk of intestinal toxicity (142;146), which may be reduced if pelvic radiation is delivered by IMRT (147;148).

### **1.1.10 Organ-specific morbidity and health-related quality of life (HRQoL) after curatively intended external beam radiotherapy**

#### *The price for cure*

Faced with several treatment options expected to have similar curability rates, the posttreatment side effects are important measures for patients and clinicians to consider when choosing the treatment modality. The potential organ-specific side effects following curative treatment include impaired urinary, bowel, and sexual function. Furthermore, secondary cancers may develop following radiotherapy (149;150). Endocrine effects from adjuvant HT also add to the total load of morbidity (90;91). In addition to specific treatment-related or

disease-related effects, cancer and its treatment may influence other aspects of a person's well-being, frequently referred to as HRQoL. Side effects and HRQoL can be assessed by physicians using predefined scales (151) or by the patients using validated instruments for self-reported outcomes (152).

Randomized studies have not compared the side effects from radical prostatectomy and radiotherapy. The available data indicate a higher risk of erectile dysfunction and urinary incontinence following prostatectomy and a higher risk of irritative urinary symptoms and bowel dysfunction following radiotherapy (153;154). Brachytherapy is probably associated with less rectal toxicity and more urinary toxicity compared with external beam radiotherapy (153). These assumptions may not be representative for individual settings because of the large variety in patient selection and treatment conditions. Pelvic morbidity after curative local treatment may be confounded further by age-dependent comorbidity, disease progression, or HT. Therefore, to estimate the proportion of morbidity that is caused directly by a specific local treatment, the optimal trial would have to include baseline data and a randomized control group of patients who receive no active treatment. No such study has been published. Therefore, the published estimates of risk for specific complications carry great uncertainty. The following section addresses the side effects related directly to external beam radiotherapy.

### *Urinary function*

Subsequent to radiotherapy, acute inflammatory prostatocystitis may give rise to symptoms such as increased frequency, urgency, incontinence, retention, hematuria, and dysuria (155). These symptoms are often transitory, but consecutive damage of the urothelial cells may lead to consequential late side effects such as bladder fibrosis, strictures, sphincter damage, hemorrhagic cystitis/urethritis, and irritative cystitis/urethritis. The most common urinary symptom following radiotherapy is probably increased frequency or urgency (155). Late genitourinary toxicity following radiotherapy appears to be relatively consistent over the first few years after radiotherapy (146;156). In a long-term longitudinal study, the self-reported incontinence on a group level was similar at the four- and eight-year follow-up, but increased significantly at the 15-year follow-up, without a similar increase in incontinence among controls from the general population (157).

### *Bowel function*

Radiotoxic injury of the rectum, sigmoid colon, and small intestine may lead to symptoms such as fecal leakage, flatulence, rectal bleeding, diarrhea, painful bowel movements, rectal urgency, increased frequency, tenesmus, and mucus discharge (158). Acute symptoms that occur during radiotherapy usually resolve, but long-term problems also occur (159). A recent longitudinal study found that late gastrointestinal symptoms often fade during the first five years after radiotherapy (146). Another study found no difference in self-reported bowel symptoms after radiotherapy for PCa at the four, eight, and 15-year follow-up, but the patients had significantly more symptoms compared with controls from the general population at all time-points (160). The risk of late bowel symptoms depends on dose (158), field size (146;161), and patient-related factors such as earlier gastrointestinal symptoms and abdominal surgery (162). Appropriate focus on conformality, and neoadjuvant HT (135) may allow for dose escalation without an unacceptable increase in gastrointestinal complications.

### *Sexual function*

Erectile dysfunction may be caused by radiation to the neurovascular bundles, internal pudendal arteries, and the proximal penile structures such as the crura of the corpora cavernosa and the penile bulb of the corpus spongiosum (163). The prevalence of erectile dysfunction following radiotherapy increases gradually for the first five years (164) most likely because of gradual fibrosis of the small vessels. Whether the radiotoxic process continues after five years is not clear. Fransson and Widmark found similar prevalences of erectile dysfunction at four and eight years of follow-up in a longitudinal study, but there was a tendency towards more general sexual problems at the later time (156).

A review of articles that specifically addressed the risk of erectile dysfunction after radiotherapy found rates of 8–85% (165). Thus, it is impossible to estimate risk exactly based on the literature. Sildenafil was effective in approximately 50% of patients with erectile dysfunction after radiotherapy in a placebo-controlled, double-blind trial (166).

#### **1.1.11 Self-reported outcomes**

Self-reported multidimensional HRQoL assessment by validated instruments may provide knowledge about the patient's own perception of health effects of cancer and its treatment that may differ from the physician's views (167). In addition, physician-reported organ-specific symptoms may differ from the patient's self-reported symptoms, as shown following pelvic radiotherapy for cervical cancer (168). Instruments for self-reported HRQoL and specific

symptoms may be generic, cancer specific, or cancer type specific (152). Longitudinal studies measure changes within self-reported outcomes over time; for example, in relation to specific interventions (164). Cross-sectional studies may provide useful information if the results are compared with those of reliable control groups; for example, with normative data from the general population (169-171).

Several studies have reported that generic HRQoL outcomes are similar between data from PCa patients following definitive radiotherapy and normative data from the general population (68;157;164;172;173). This may reflect that generic HRQoL instruments might be insensitive to cancer- or treatment-related symptoms. Furthermore, a response shift may occur among cancer patients (174) as they accept disease- or treatment-related symptoms as inevitable and do not integrate them into their perception of general quality of life. These assumptions were supported by a cross-sectional single-institution study at the NRH of patients at least one year following radical prostatectomy or radiotherapy (172). Specific organ-related post-treatment morbidity such as urinary incontinence, erectile dysfunction, and bowel distress did not independently predict global HRQoL in a multivariate analysis. On the other hand, poor urinary function, urinary bother, sexual function, and sexual bother were independently associated with worse generic HRQoL outcomes two years after diagnoses of PCa in a large American population-based study (173).

## **1.2 Disseminated tumor cells (DTCs)**

### **1.2.1 Minimal residual cancer**

With a few exceptions, (e.g. testicular cancer), distant metastases usually exclude curative treatment of solid malignancies. M staging is the single most important prognostic factor at diagnosis. However, many M<sub>0</sub> patients experience metastatic relapse following local treatment of the primary tumor (58). The principle explanations are unsuccessful local eradication of the tumor or the existence of occult metastatic tumor cells that have been shed from the primary tumor prior to primary treatment. The detection of tumor cells by sensitive methods such as ICC and polymerase chain reaction (PCR) have enhanced our understanding of the metastatic process and have resulted in comprehensive models for malignant spread and development of metastases (175). The cells detected by these methods are frequently referred to as “micrometastases”, “occult tumor cells”, or “minimal residual cancer”, which all refer to metastatic cancer that cannot be detected by routine diagnostic procedures (176). According to contemporary definitions, DTCs refer to tumor cells that are detected in the BM, and circulating tumor cells (CTCs) refer to tumor cells that circulate in the peripheral blood. Isolated tumor cells (ITCs) refer to minimal residual cancer cells in general.

In theory, several clinical applications of such approach are possible. Detection of ITCs in M<sub>0</sub> patients at diagnosis may predict the development of distant metastases despite successful local tumor eradication. If so, detection of the cells may identify high-risk patients who may not benefit from local therapy alone, but who may benefit from adjuvant therapy in addition to local eradication of the tumor. Furthermore, patients without ITCs may be spared for adjuvant treatment. Characterization of the cells may also guide adjuvant therapies directed towards specific targets within the cells. In posttreatment monitoring, detection of isolated tumor cells may be an early surrogate marker of treatment failure in patients characterized as “tumor free” by conventional biochemical and diagnostic methods.

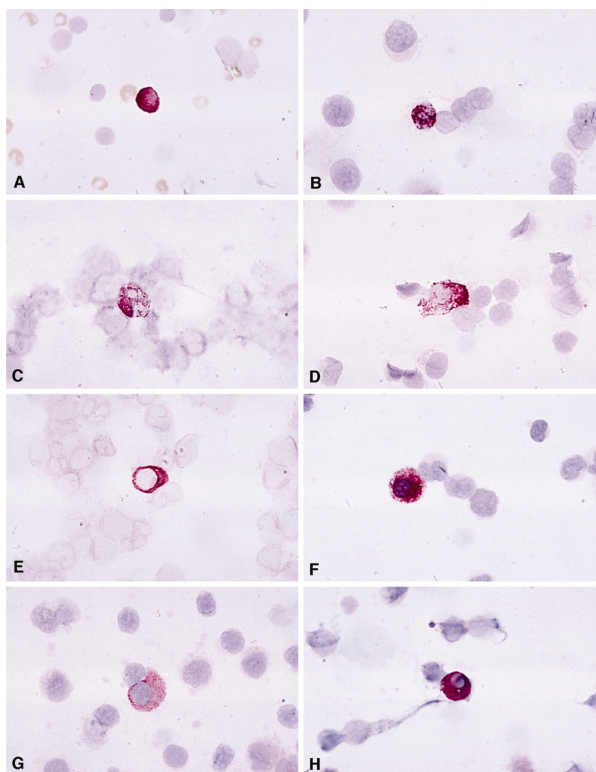
### **1.2.2 Technological aspects**

ITCs are detected mainly by ICC using monoclonal antibodies or by PCR with cancer-specific or tissue-specific transcripts. The main advantage of ICC is maintenance of cell morphology and thus the possibility of further biological and morphological characterization of the cells with consequent exclusion of false-positive immunostained noncancer cells (Fig 1.2) (177). Direct quantification of ICC-detected ITCs can be performed. Among several factors, the lack of true cancer-specific molecular targets remains a problem for the specificity of PCR-based

techniques (178). Furthermore, quantification of tumor cells is difficult, although contemporary real-time PCR partly may solve this problem (179).

Most assays to detect minimal residual cancer in peripheral blood or the BM include enrichment of mononuclear cells (lymphocytes and monocytes) by density centrifugation (180) because tumor cells are usually trapped in the mononuclear cell layer. In practice, the amount of mononuclear cells that can be investigated following density centrifugation is restricted to the  $10^6$  level with ICC and the  $10^7$  level with PCR. The number of mononuclear cells investigated by ICC can be increased to the  $10^7$  level following further enrichment of tumor cells by negative and/or positive immunomagnetic separation techniques that apply paramagnetic microparticles coated with antibodies to leucocytes or tumor cells (181;182).

The application of monoclonal antibodies directed against cytokeratins has become the mainstay for ICC detection of DTCs in the BM from patients with carcinomas (183). Cytokeratins are proteins in the cytoskeleton of epithelial cells that are not normally expressed in hematopoietic cells. Anti-PSA antibodies have also been applied for detecting PCa cells, but these are less sensitive than anticytokeratins (184). The most frequent anticytokeratin antibodies in use for DTC detection are AE1/AE3 (185), A45/B-B3 (186), and CK2 (187). AE1/AE3 and A45/B-B3 are assumed to be pancytokeratin antibodies (because they react with several subtypes of cytokeratins), whereas CK2 reacts only with cytokeratin 18. The importance of excluding false-positive cells by standardized morphological evaluation of all immunostained cells has been emphasized (177).



**Fig. 1.2**

Examples of morphological features of disseminated breast cancer cells categorized as tumor cell (A and B), uninterpretable cell (C and D), probable hematopoietic cell (E and F), and hematopoietic cell group (G and H).

Reprinted from Clinical Cancer Research Vol. 10. The prognostic value of isolated tumor cells in bone marrow in breast cancer patients: Evaluation of morphological categories and the number of clinically significant cells.

Naume B, Wiedswang G, Borgen E, Kvalheim G, Karesen R, Qvist H, Janbu J, Harbitz T, Nesland JM. Page 3093. Copyright (2004), with permission from the American Association for Cancer Research, Inc.

### 1.2.3 Clinical findings and prognostic impact

The clinical impact of detecting minimal residual cancer has been studied most extensively in breast cancer: In a pooled analysis of 4703 patients with a median follow-up of 62 months, a positive DTC status in the BM at diagnosis was independently associated with increased risk of death from any cause, death from breast cancer, disease recurrence, and distant metastases (188). Positive DTC status at diagnosis was associated with large tumor size, high histopathological grade, lymph node metastases, and negative hormone receptor status. Smaller studies have demonstrated a correlation between DTCs in the BM at diagnosis and treatment failure in other solid tumors such as colorectal cancer (189), lung cancer (190;191), malignant melanoma(192), osteosarcoma (193), and PCa (70;72;76).

Compared with DTCs in the BM, finding of CTCs in peripheral blood is generally less frequent (73;74;194), and seems to be less predictive of the clinical course (194). The reason may be that tumor cells circulate in the blood only for a limited time before settling at distant sites. Interestingly, the predictive impact of DTC detection was reduced in cohort of patients

when the number of mononuclear cells investigated was increased from  $2 \times 10^6$  to  $6 \times 10^6$ . The explanation may be that a certain load of DTCs is necessary for the development of distant metastases.

The appearance of DTCs in the BM following primary therapy for breast cancer (195) or PCa (73) seem to predict subsequent clinical progression, suggesting a possible role for monitoring patients during follow-up. In addition, quantification of CTCs by a commercial automated method (CellSearch) can predict the clinical course in both metastatic breast cancer (196) and castrate-resistant PCa (197).

#### **1.2.4 Disseminated prostate cancer cells in the BM**

Because about 90% of PCa patients with distant metastases have skeletal lesions (30), the BM seems to be an appropriate site to look for minimal residual cancer. This section presents published studies that have detected DTCs in the BM of PCa patients except for those studies included in the current thesis (70;71). The inconsistent results in these studies probably reflect different methods and lack of standardization.

Oberneder et al assessed DTC status in 84 patients with  $T_{1-4}N_{0-1}M_{0-1}$  PCa by ICC using the monoclonal antibody CK2 (198). The prevalence of positive DTC status was 36%. Compared with DTC-negative patients, DTC-positive patients had a significantly higher risk of extracapsular tumor extension, distant metastases, and unfavorable histological grade. In patients with well- or moderately differentiated tumors (WHO grading), DTC-positive patients had a higher risk of lymph node metastases.

Pantel et al used the same method and found DTCs in 55% of 44  $cT_{3-4}pN_XM_0$  patients (199). BM findings were not associated with tumor volume, the GS, or PSA concentration. The same research group performed repeated BM aspirations in 36  $T_{3-4}pN_XM_0$  patients who received HT (200). The prevalence of positive DTC status was 58% before and 17% after 3–5 months of complete androgen blockade, indicating that DTC detection may be used as a surrogate marker of the response to treatment.

Using antibodies against cytokeratins 8 and 18, Mueller et al detected DTCs in 45% of 67 patients with  $cT_{1-4}N_{0-1}M_0$  PCa; 37 of these patients had received HT before BM aspiration (184). Positive DTC status was independent of PSA concentration, T-category, and HT. Only 12% of cytokeratin-positive cells coexpressed PSA according to a dual staining procedure. Most of the cytokeratin-positive cells were cytogenetically aberrant according to fluorescence in situ hybridization quantification of chromosomes 1, 7 and 8, implying a malignant origin of these cells.



Weckermann et al detected DTCs using CK2 before radical prostatectomy in 219 patients with pT<sub>1-3</sub>pN<sub>0</sub>M<sub>0</sub> PCa (75). The prevalence of positive DTC status was 24%. Positive DTC status was not associated with the GS, pT category, perineural invasion, lymphatic invasion, or pretreatment PSA concentration, or with lymph node status when 47 N<sub>1</sub> patients were included. Ploidy was determined in 58 of the patients but was not associated with DTC status. Because cytokeratin 18 is probably downregulated in adenocarcinomas (201), the same research group performed a second study (76) that included 82 patients from the first study, six of whom had regional lymph node metastases. BM samples from these patients were incubated with the pancytokeratin antibody A45/B-B3 in addition to CK2. The prevalences of positive DTC status were 24% for CK2 and 40% for A45/B-B3, but the DTC status was not associated with other prognostic variables. A45/B-B3-positive DTC status, but not CK2-positive DTC status, independently predicted PSA recurrence after four years median follow-up.

In 2003, Lilleby et al published the first results of DTC detection in PCa patients referred to the NRH for radiotherapy (73). The pancytokeratin antibodies AE1/AE3 identified positive DTC-status before definitive radiotherapy in 21% of 66 cT<sub>1-4</sub>pN<sub>0</sub>M<sub>0</sub> patients, but positive status was not associated with the GS, cT category, or pretreatment PSA. The Pre-radiotherapy DTC status did not predict clinical progression-free survival following radiotherapy at median five years of follow-up. BM was also aspirated in 60 of the patients 25–57 months after radiotherapy. Positive post-radiotherapy DTC status was found in 20% and was associated with an increased risk of clinical progression compared with patients with negative post-radiotherapy DTC status, supporting that DTC detection may be useful for monitoring the effects of therapy.

Kollermann et al used A45/B-B3 to detect DTCs in the BM before neoadjuvant complete androgen blockade followed by prostatectomy in 193 cT<sub>1-3</sub>pN<sub>X</sub>M<sub>0</sub> PCa patients (72). Positive DTC status was found in 45% but was not significantly associated with pretreatment PSA concentration, cT category, pT category, or the GS in needle biopsies or prostatectomy specimens. However, positive DTC status was an independent predictor of PSA recurrence after a median 61 months of follow-up.

Wood et al applied an RT-PCR assay that targeted PSA mRNA in the BM before prostatectomy in 86 pT<sub>1-3</sub>pN<sub>0-1</sub>M<sub>0</sub> PCa patients (202). Positive DTC status was found in 45% of patients and was associated with extraprostatic disease in the surgical specimens and pretreatment PSA  $\geq 10\mu\text{g/L}$ , but not with the GS ( $< 7$  versus  $\geq 7$ ) in needle biopsies or surgical specimens. Positive DTC status predicted PSA recurrence in a univariate analysis

after a mean follow-up of 15 months, but not in multivariate analysis when PSA concentration at diagnosis was included. The same research group later reported that 96% of 46 patients with PSA mRNA in their BM also had ICC-detected cytokeratin-positive DTCs in their BM (203). Double staining with an antibody directed against the proliferation marker Mib-1 was performed in a subgroup of 38 patients and showed positive findings in 68% of these, indicating that these cells were in the active cell cycle. In a third publication, PSA mRNA was found in 79 of 244 (32%) patients with cT<sub>1-2</sub> PCa before prostatectomy (204). Double ICC staining for cytokeratins and Mib-1 was performed on 58 of the RT-PCR-positive patients. Cytokeratine-positive cells were found in all patients and Mib-1 positive cells were found in 36% of the patients. The presence of proliferating cells was associated with the GS in the prostatectomy specimens but not in needle biopsies or with other variables. Patients with proliferating DTCs had a significantly higher risk of PSA recurrence compared with patients with non-proliferating DTCs.

Several papers on DTCs in the BM and peripheral blood of PCa patients have been published from the University of Washington in Seattle (74;181;205-208). Both RT-PCR and ICC have been applied. Isolation of tumor cells and increased sensitivity of their assays have been achieved using a combination of positive and negative immunomagnetic selection techniques (181). After enriching the tumor cell fraction, the group found that 49% of the DTCs in the BM expressed Telomerase (208). A later publication reported on PSA RT-PCR and ICC using the antibody BerEP4 against the epithelial marker EpCam in BM aspirates (74). Before prostatectomy, 49 of 154 (32%) were positive for PSA mRNA and 121 of 162 (75%) were positive for BerEP4. Eighty-six (71%) of the BerEP4 positive patients had PSA-expressing cells in the BM, which were identified by positive findings on PSA RT-PCR or PSA ICC. In a subgroup of 32 patients who had no PSA recurrence at least one year after prostatectomy, 12% were positive for PSA mRNA and 47% for BerEP4, implying the existence of dormant tumor cells in these patients. In the same study, a control group included 27 men who were assumed to be without PCa based on PSA concentration < 2.5 µg/L. Among these, one (4%) had PSA mRNA in his BM and three (11%) had BerEP4-positive cells.

### **1.2.5 Minimal residual prostate cancer in peripheral blood and lymph nodes**

#### *Peripheral blood*

Before settling in the BM, tumor cells must migrate through the bloodstream. Tumor cells can also circulate in the blood secondarily to metastases in distant organs. Because a venous puncture is more convenient than a BM aspiration, several studies have identified CTCs in the peripheral blood of PCa patients. Many of these studies have applied RT-PCR as the detection method. A positive finding before prostatectomy in some studies been associated with biochemical progression (209-212) and in one study with development of distant metastases (213). However, these findings have not been supported by other studies (206;214;215). Inconsistent results have also been reported on the relationship between CTCs and other risk factors. Shariat et al concluded that positive PSA RT-PCR in peripheral blood 6–8 weeks after, but not before prostatectomy predicts PSA-based failure (216). In progressive castrate-resistant PCa, quantification of CTCs by the automated CellSearch system can provide prognostic information (197).

#### *Lymph nodes*

In addition to unrecognized hematogenous dissemination, occult spread to regional lymph nodes probably contributes to treatment failure after radical local treatment. Several studies have shown that occult tumor cells can be detected by ICC or RT-PCR in lymph nodes that are judged to be free from cancer by standard pathological evaluation (26;27;210;211;217-226). Two relatively large prospective studies with long-term follow-up have shown that positive findings are predictive of clinical survival endpoints following prostatectomy. Shariat et al investigated lymph node specimens from 199 prostatectomized patients classified as pT<sub>3</sub>pN<sub>0</sub> following extended lymph node staging (27). Occult tumor cells were detected by RT-PCR targeting the prostate-specific human glandular kallikrein (hK2) and ICC with antibodies against PSA, cytokeratin, and hK2. The lymph node status was positive for tumor cells in 13% by ICC and 20% by RT-PCR. Positive findings by both methods were associated with increased risks of PSA recurrence, development of distant metastases, and death from PCa. Pagliarulo et al included 274 pT<sub>3</sub>N<sub>0-1</sub> patients who had been treated by radical prostatectomy and surgical lymph node staging and followed them for 13 years, median (26). Occult tumor cells were detected by ICC in lymph nodes from 24 of 180 patients (13%) who were judged to be pN<sub>0</sub> following routine pathological evaluation. Patients with pN<sub>0</sub> disease with occult lymph node metastases had a higher risk of PSA recurrence and death from any cause compared with

pN<sub>0</sub> patients without occult lymph node metastases. The recurrence rates for pN<sub>0</sub> patients with occult lymph node metastases and pN<sub>1</sub> patients were similar.

## Chapter 2: The current thesis

### 2.1 Introduction

The work presented in this thesis began in October 2004. At that point, dose escalation to 74Gy and adjuvant castration-based HT for high-risk patients (94) was the standard treatment when radical radiotherapy was given for PCa at the NRH. From 1989 to February 1996, radiotherapy was given as monotherapy with doses  $\leq 70$ Gy. Most patients eligible for radiotherapy were not considered to be candidates for surgical prostatectomy, because of either locally advanced tumor growth or other factors such as comorbidity or age. Radical radiotherapy was only offered to patients who were considered to be without lymph node metastases based on surgical staging. The five-year clinical outcomes from those who were treated in that period have been published (227) as well as the HRQoL findings at least one year after radiotherapy (172). Because of the long natural history of PCa and because relatively few studies had been published in 2004 including 10-year clinical outcomes following radiotherapy for pN<sub>0</sub> PCa (228-230), a repeated follow-up of the patients was needed. *Paper I* comprises the mature 10-year clinical outcomes for all 203 patients who received definitive radiotherapy at the NRH from 1989 to February 1996 and the self-reported HRQoL outcomes in the surviving patients (68).

During 1996–2002, definitive radiotherapy was usually given at the NRH within the framework of the seventh trial of the Scandinavian Prostate Cancer Group (SPCG-7), which compared AA with or without radiotherapy for locally advanced or localized high-grade PCa (105). Comparison of clinical outcomes and HRQoL among these patients has been performed by the SPCG-7 study group and was outside the scope of this thesis. However, to expand the knowledge about the consequences of combined radiotherapy and long-term AA, there was a need to compare HRQoL between patients who received such treatment and age-matched controls from the general population. Increases in serum testosterone and estradiol concentrations have been reported following one and two years of AA treatment (99;231). Hormonal changes in patients who have used AA for a longer time had not been reported before the planning of this thesis. A prolonged increase in serum estradiol concentration may be important because of the possible increase in the risk of cardiovascular morbidity (93). In *paper II*, self-reported outcomes at the five-year follow-up in patients included in the radiotherapy + AA arm at the NRH were compared with self-reported outcomes from age-

matched controls in the general population. Longitudinal changes in sex hormone concentrations during the five years of AA treatment were also explored.

The “micrometastases project” was established at the NRH early in the 1990s to detect and characterize single metastatic tumor cells in patients without overt metastases. The theoretical rationale was that such cells might represent early evidence of metastatic disease and, if so, would provide prognostic information and be potential targets for adjuvant therapy. ICC has been used for DTC detection, and the project has made important contributions to improving and standardizing the methods (177;182;232-235). The project has also contributed greatly to the comprehensive body of evidence that DTCs in the BM predict treatment failure in breast cancer (195;236). Between 1994 and 2007, PCa patients were asked to donate BM samples for DTC detection before and after radiotherapy. Preliminary results showed a relationship between the presence of DTCs after radiotherapy and clinical progression-free survival but no relationship between the presence of DTCs before treatment and five-year clinical outcomes (73). Weckermann et al showed previously that DTCs detected by ICC predict PSA-based recurrence following radical prostatectomy (76). In both studies, the detection of DTCs was independent of the standard prognostic factors PSA concentration, the GS, and T-category. The rationale for *paper III* (70) was based on the hypothesis that expansion of the patient group would show an association between DTCs and established prognostic factors, and that longer follow-up would show an association between DTCs and the development of distant metastases following radiotherapy. In *paper IV* (71), the prevalence of DTCs in the BM after definitive radiotherapy with or without HT were investigated.

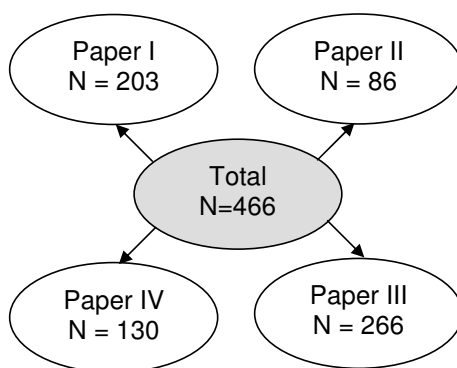
## **2.2 Study aims**

Based on the background outlined above, the current thesis was motivated by the following study aims:

1. To assess mature 10-year clinical outcomes following definitive radiotherapy  $\leq 70$  Gy as monotherapy for PCa.
2. To assess long-term self-reported outcomes following definitive radiotherapy as monotherapy for PCa and to compare the findings with normative data from the general population.
3. To assess self-reported outcomes in PCa patients following definitive radiotherapy + long-term adjuvant treatment with an oral nonsteroidal AA and to compare the findings with those in age-matched controls from the general population.
4. To assess longitudinal changes in sex hormone concentrations in PCa patients who have used an oral nonsteroidal AA continuously for five years.
5. To assess the prevalence of DTCs in the BM of patients with untreated M<sub>0</sub> PCa using a standardized ICC method and to explore the possible associations between DTC status before treatment and clinical, biochemical, and histopathological variables.
6. To explore whether the appearance of DTCs in the BM of PCa patients before definitive radiotherapy predicts clinical outcomes.
7. To assess the prevalence of DTCs in the BM of clinically progression-free PCa patients after definitive radiotherapy with or without adjuvant HT.

## 2.3 Patients and methods

The current thesis comprises four substudies represented by papers I–IV. A total of 466 men were included with some overlap between the substudies (Fig. 2.1). They had all been referred to the Department of Oncology, NRH because of newly diagnosed M<sub>0</sub> PCa during 1989–2004.



**Fig. 2.1.** Distribution of patients between substudies.

### 2.3.1 TNM staging

The clinical status was T<sub>1–4</sub>pN<sub>0</sub>M<sub>0</sub> in all patients except 24 who were T<sub>1–4</sub>pN<sub>X</sub>M<sub>0</sub>. The latter patients were considered to have a low risk of lymph node metastases based on pretreatment PSA concentration < 10 µg/L. Pelvic lymphadenectomy had been performed in all the other patients to verify the pN<sub>0</sub> status, which was required to receive the curatively intended radiotherapy. The cT-category had been assessed by digital rectal examination. Chest X-ray and technetium bone scan, with supplementary skeleton X-ray or MRI if indicated, had been performed to confirm the M<sub>0</sub> status in all patients.

### 2.3.2 Histopathological parameters

At referral, all PCa diagnoses were confirmed routinely by histopathological review of the patients' biopsies at the Department of Pathology, NRH. The patients in paper I received treatment in 1989–1996, and their biopsies were graded originally according to WHO grading system (40). These had been regraded using the GS in a previous follow-up study (227). For papers III and IV, all available biopsies were regraded to provide a GS by one experienced pathologist using new guidelines (34). For paper III, the “percentage of Gleason pattern 4/5”, “percentage positive biopsies”, and “percentage tumor length in all cores” (35;36) were



assessed from needle biopsies. When there were two or more separate foci of cancer in the same needle, the uninvolved tissue between the foci was included in the assessment of “percentage tumor length in all cores”.

### **2.3.3 Biochemical parameters**

An in-house time-resolved fluoroimmunoassay for PSA was applied until June 2006 (237), and the AutoDELFIA ProStatus PSA Free/Total kit (PerkinElmer, Waltham, Massachusetts) was used after June 2006. The two methods are identical in format and calibration, and both have a detection limit of 0.05 µg/L.

Testosterone concentration was measured with a Spectria-coated tube (Orion Diagnostica, Espoo, Finland). The concentrations of estradiol and sex hormone-binding globulin (SHBG) were measured using the Autodelphia method (Wallac, Turku, Finland), and the concentrations of follicle stimulating hormone (FSH) and LH were measured by the Access method (Beckman, Fullerton, California). The coefficients of variation were less than 10% for each method. The reference ranges were testosterone 8–35 nmol/L, estradiol < 0.13 nmol/L, SHBG 25–65 nmol/L, LH < 12 IU/L and FSH < 12 IU/L.

### **2.3.4 Treatment**

#### *Radiotherapy*

Most of the included patients received curatively intended external beam radiotherapy with target-doses of 64–74Gy depending on the year of treatment. The standard dose at the NRH was 66 Gy until 1996, 70 Gy in 1996–2000 and 74 Gy thereafter. A four-field box technique (two opposing anterior–posterior fields and two opposing lateral fields) was used. In 1989–1994, multiple rectangular and triangular shielding blocks based on two-dimensional CT imaging were used. In August 1994, a modification was introduced that used a multileaf collimator and a three-dimensional CT planning system (Helax, Uppsala, Sweden) (Fig. 1.1). The gross tumor volume included the prostate and seminal vesicles. To account for microscopic disease, the clinical target volume comprised the gross tumor volume and an additional margin of 10 mm. The planning volume included the clinical target volume and a margin of another 10 mm to account for organ motion and setup errors. In addition, the irradiation field encompassed the planning volume and a margin of 5 mm to account for the penumbra so that the irradiation field in general added 25 mm to the gross tumor volume except that the posterior field margin towards the rectum added a maximum of 20 mm to the gross tumor volume up to 1996 and 10–15 mm thereafter. For tumors not involving the

seminal vesicles, the gross tumor volume was reduced to encompass only the prostate after 50 Gy. All patients received megavolt photon energy, 15 MV, with daily fractions of 2 Gy, five days per week. The dose was prescribed on the central axis at the projected center of the target volumes, and from 1992 with regard to the reference point in the International Commission on Radiation Units and Measurements report number 50 (238).

### *Hormone treatment*

From March 1996, most radiotherapy-treated patients received neoadjuvant, concomitant, and adjuvant HT if they had cT<sub>3-4</sub>, a PSA concentration > 20 µg/L, or intermediate or poorly differentiated tumors (WHO grade 2 or 3). During 1996–2002, patients had been included in the SPCG-7 study (105). These patients received neoadjuvant complete androgen blockade for three months (flutamide 250 mg  $\times$  3 + leuporeline 3.75 mg/month) followed by definitive radiotherapy and continuous nonsteroidal AA medication with flutamide 250mg  $\times$  3 or bicalutamide 150 mg  $\times$  1, or the same AA treatment without radiotherapy. Patients not included in the SPCG-7 study received medical castration with a GnRH agonist for two years (three months of neoadjuvant) or later for three years (six months of neoadjuvant). An oral nonsteroidal AA was given in the first month to avoid flare symptoms. The latter treatment became standard when the SPCG-7 trial was closed to inclusion.

### **2.3.5 Follow-up**

Following primary therapy, patients were followed regularly by their local urologist in cooperation with their family doctor and the Department of Oncology, NRH. Most of the patients revisited the Department of Oncology's outpatient ward once or more during follow-up for clinical evaluation. All patients included in papers II and IV were examined at the Department of Oncology at the last follow-up in these substudies.

### **2.3.6 Collection of clinical data**

Clinical data relating to the pretreatment variables were collected from patients' files at the NRH. Survival status on the cutoff date of each of the two follow-up studies in papers I and III was retrieved from the Central Population Registry based on the unique 11-digit personal identification number. The cause of death was determined retrospectively by reviewing the hospital records and by contact with the responsible local clinicians. These sources also provided information on eventual date and type of progression.

### **2.3.7 Definitions of clinical outcome**

The endpoints overall death and overall survival were related to death from any cause. Cause-specific death and cause-specific survival related to death due to PCa, death due to complications from treatment of PCa, or death from unknown causes in patients proven to have progressive castrate-resistant PCa. Clinical progression was defined as local or distant progression. Distant progression was defined as radiological evidence of distant metastases and local progression as clinically detectable growth of the prostate gland or loco-regional lymph nodes. In accordance with the Houston definition, PSA recurrence (called biochemical failure in paper III) was defined as an increase in PSA concentration of at least 2 µg/L above the nadir (defined as the last nonrising value) (53). In addition, any secondary therapeutic intervention based on increasing PSA concentration before the criterion was fulfilled was scored as PSA recurrence at the time of intervention. PSA recurrence was not evaluated as an endpoint in paper I because routine follow-up did not include assessment of PSA concentration during 1989–90.

### **2.3.8 Instruments for self-reported outcomes**

The following validated questionnaires were applied in papers I and II (see appendix). They were chosen because of satisfactory psychometric properties and available normative data.

#### *The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Instrument (QLQ-C30)*

The QLQ-C30 measures general HRQoL in cancer patients and comprises 30 items; 28 of these are scored on a 4-point Likert scale (239;240). Five multi-item functional scales measure physical function (five items), role function (two items), emotional function (four items), cognitive function (two items), and social function (two items). Nine symptom scales/items measure fatigue (three items), nausea and vomiting (two items), pain (two items), dyspnoea (one item), insomnia (one item), appetite loss (one item), constipation (one item), diarrhea (one item), and financial difficulties (one item). In addition, global health status and QoL are assessed by two separate items scored on a 7-point Likert scale ranging from very poor to excellent. The scores are combined into a global HRQoL scale (called GlobQL in paper I). All raw scores are linearly transformed into scales ranging 0–100 (241). Higher scores in global HRQoL and the functioning scales reflect better global HRQoL and better function. Higher scores in the symptom scales/items reflect more symptoms. The Norwegian version of the QLQ-C30 has shown satisfactory internal consistency in a study that provided

the normative data from the Norwegian general population (170). The QLQ-C30 version 3.0 was used in paper I.

#### *The Short Form-36 (SF-36)*

The SF-36 is a widely used instrument for assessing HRQoL in general (242). It comprises the eight multi-item HRQoL domains physical functioning (10 items), role limitations due to physical problems (four items), bodily pain (two items), general health perception (five items), vitality (four items), social functioning (two items), role limitations due to emotional aspects (three items), and mental health (five items). These are combined into two summary scores: the physical component summary and the mental component summary (243).

Dimension scores are transformed to a scale ranging 0–100 (244). The summary scores are transformed by linear T-score transformation so that the general population has a mean of 50 and standard deviation of 10 (245). Higher domain scores represent better function. The Norwegian version has good internal consistency (246). The SF-36 version 1.0 was used in paper II.

#### *The Brief Male Sexual Function Inventory (BSFI)*

The BSFI comprises 11 items that are combined into three multi-item functional domains: sexual drive (two items), erections (three items), and ejaculation (two items); one domain for sexual problem assessment (three items); and one single-item domain that measures overall sexual satisfaction (247). The items are scored on a five-point Likert scale (0–4). Higher scores represent better function, fewer problems, and a higher degree of satisfaction with one's sex life.

Based on face validity, the respondents were considered to have complete *erectile dysfunction* if they responded “not at all” to question #4: “Over the past 30 days, when you had erections how often were they firm enough to have sexual intercourse?” They were considered to have *lack of sexual drive* if they responded “low” to question #2: “During the past 30 days, how would you rate your level of sexual drive?” *Sexual dissatisfaction* was identified if the patient was “very dissatisfied” or “mostly dissatisfied” with his sex life the past 30 days (question #11).

The Norwegian version of the BSFI showed good internal consistency in a recent study of Norwegian men from the general population, which provided the normative mean scores in 10-year age categories for paper I (171). Published American normative data

provided the age-adjusted responses to single items (248). The BSFI was used in papers I and II.

#### *The University of California, Los Angeles Prostate Cancer Index (PCI)*

The PCI addresses general and organ-related HRQoL in patients treated for PCa (249). Scores are linearly transformed into scales ranging 0–100, which are based on raw scores from 3–6 point Likert-scales. Higher scores reflect better function and less bother. Normative data for American men without PCa have been published (250).

For paper I, domains from the PCI relating to urinary function (five items), urinary bother (one item), bowel function (four items), and bowel bother (one item) were used. The bowel function domain of the PCI does not cover fecal control and the use of pads because of fecal leakage. Two ad hoc questions were added to cover these aspects, as fecal leakage can be an important side effect of radiotherapy (Table 3, paper I).

#### *The Hospital Anxiety and Depression Scale (HADS)*

The HADS was developed to measure symptoms of depression and anxiety in patients with somatic illnesses (251). The instrument comprises 14 items scored on a 4-point Likert-scale (0–3). The scores for seven items are added up to give the depression score (0–21), and seven different items are summed to give the anxiety score (0–21). Caseness of depression and anxiety are defined as a score of  $\leq 8$  within each scale. This cutoff point provides the best balance between sensitivity and sensibility in most studies (252). The basic psychometric properties of the Norwegian version are considered satisfactory based on a large sample from the Norwegian general population (253). The HADS was used in paper II.

#### *The Fatigue Questionnaire (FQ)*

The FQ measures mental (four items) and physical (seven items) features of fatigue on a 4-point Likert-scale (0–3) (254). Mental fatigue and physical fatigue scores are derived from the sums of the scores within each scale (0–12 and 0–21, respectively). Total fatigue is the sum of all 11 items (0–33). Two additional questions are asked about the duration and extent of the symptoms. Caseness of chronic fatigue is defined as the sum of dichotomized scores (0–1 = 0, 2–3 = 1) of  $> 4$  and duration of six months or more (255). The Norwegian version was shown to have good internal consistency in a study of the Norwegian general population (256). The FQ was used in paper II.

### *Missing responses*

Missing responses within a domain were replaced by the mean of the completed items in that domain if at least half of the items were scored. In other cases, domain scores were considered as missing (257).

### **2.3.9 Normative data and control groups**

#### *HRQoL*

In paper I, the data for patients who had been treated by definitive radiotherapy were compared with published, age-adjusted normative data from the general population (170;171;248;250). In paper II, data for age-matched control cohorts were retrieved from the general Norwegian population: Using public data lists, the NRH had previously collected several samples of normative data from the general population to be used for comparisons with cancer patients. In 2004, a sample of 2100 men aged 50 to 79 years received a questionnaire including the BSFI, and 756 (36%) responded. In 2005, a sample of 1380 males of the same age range received a form including the HADS and the FQ, and 473 (34%) responded. Respondents reporting cancer were excluded. Based on these samples, the BSFI data for two age-matched controls within the same five-year category for each patient were retrieved, and the HADS and FQ data for one control for each patient were retrieved. Normative cases for the SF-36 were selected from the Living Condition Study of 2002 performed by Statistics Norway on a representative sample of the population. From 1028 men aged 50 to 79 years, we selected two cancer-free controls per patient, also age-matched in five-year categories.

#### *Survival*

To compare 10-year overall survival in PCa patients treated by radiotherapy monotherapy with that of men from the general population (paper I), Statistics Norway constructed a control cohort using the following method. Three age-matched men for each nonemigrating patient were identified randomly. The eligible controls had to be alive the year radiotherapy was started in the corresponding case. The year of birth and year of death for each anonymous control were made available to the first author.

### **2.3.10 Detection of DTCs**

#### *Aspiration and preparation of BM*

Local anesthesia was applied, a small skin incision was made, and 20–30ml of BM was aspirated from one of the posterior iliac crests during the early phase of the study (paper III) and later from both sides (paper IV). Fresh aspirates were diluted 1:1 in PBS (PAA Laboratories, Pasching, Austria). Mononuclear cells were isolated by density centrifugation using Lymphoprep (Axis Shield, Oslo, Norway) and washed twice in PBS with 10% FCS (PAA Laboratories). Cytospins were made by centrifuging the mononuclear cells onto polylysine-coated glass slides, each containing  $5 \times 10^5$  cells. The slides were air-dried overnight and stored at  $-80^{\circ}\text{C}$  until immunostaining was performed.

#### *Immunostaining*

The cytopins were defrosted and fixed for 10 minutes in acetone. Four cytopins were incubated in a moist chamber with the pancytokeratin mononuclear antibodies AE1/AE3 (Chemicon International Inc.) at a concentration of 20  $\mu\text{g/ml}$  for 30 minutes. The visualization step included the alkaline phosphatase/anti-alkaline phosphatase reaction, and the slides were counterstained with hematoxylin to visualize the nuclear morphology. Another four spins were incubated with an isotype-specific irrelevant monoclonal antibody as the negative control (177). Before 1999, only one negative control spin was made. The positive control was performed by producing cytopins with BM from healthy volunteers to which cytokeratin-expressing tumor cell lines were added.

#### *Morphological characterization of immunostained cells*

The cytopins were screened manually for immunostained cells in the early phase of the study and automatically later (233). To distinguish tumor cells from immunostained hematopoietic cells, all stained cells were evaluated by an experienced pathologist using published guidelines (177). If the sample contained at least one stained cell that was judged to be a tumor cell, the BM status was regarded as DTC positive. Slides with uninterpretable cells (see Fig. 1.2) were regarded as DTC positive because the presence of these cells predicts clinical outcomes in breast cancer (235). Samples were regarded as inconclusive if they contained immunostained cells that were classified as tumor cells in both the AE1/AE3 slides and in the corresponding negative control slides. These patients were excluded from further analysis.

### **2.3.11 Statistical considerations**

#### *General statistical methods*

Statistical calculations were mainly performed using different versions of the Statistical Package for Social Science (SPSS, Chicago, IL). Continuous variables with skewed distributions were compared using the nonparametric Mann–Whitney test or Spearman’s rho for correlation analysis. Categorical variables were compared using  $\chi^2$  tests. Survival was estimated by the Kaplan–Meier method, and differences between curves were compared by the univariate log-rank test. Multivariate analysis of survival data was performed using Cox regression. The observation time ranged from the start of treatment to the date of the endpoint, the patient’s death, emigration, or the date of the last available clinical information, whichever occurred first. HRQoL scores were treated as continuous variables if a domain comprised at least two items. Single-item domains were treated as categorical variables in the statistical analysis. The internal consistency of the HRQoL scales was examined with Cronbach’s coefficient  $\alpha$ . The level of statistical significance was defined as  $p < 0.05$ , and all tests were two sided.

#### *Statistical versus clinical significance*

Statistically significant differences within HRQoL domains on a group level may or may not be of clinical relevance for the patients. Two established definitions of clinical significance were applied in the thesis. In paper I, a difference of at least 10 points on the 0–100 scale in the QLQ-C30 was regarded as clinically relevant (258). In paper II, differences in HRQoL scores were considered clinically significant if the Cohen’s effect size was  $\geq 0.40$  (259;260).

### **2.3.12 Ethical considerations**

All parts of the thesis were approved by the Regional Committee for Medical Ethics, the protocol review committee of the NRH, and the Norwegian Data Inspectorate. Written informed consent was obtained from all patients before BM sampling (papers III and IV) and in relation to the HRQoL surveys (papers I and II).



## **2.4 Summary of papers and main results**

### **2.4.1 Paper I**

The aims of this study were to evaluate long-term overall survival, cancer-specific survival, clinical progression-free survival, and HRQoL following definitive radiotherapy as monotherapy given to 203 consecutive T<sub>1-4</sub> pN<sub>0</sub>M<sub>0</sub> PCa patients at the NRH during 1989–1996. In this period, radiotherapy was considered in patients with pN<sub>0</sub>M<sub>0</sub> disease who were not suitable for radical prostatectomy because of extracapsular growth or patient-related factors such as obesity or other comorbidities. The patients were between 48 and 81 years (median, 66 years) when they started radiotherapy. The target dose was 66 Gy in 196 patients (97%), and none received neoadjuvant, concomitant or adjuvant HT. Eighty-one percent belonged to a high-risk group defined as cT<sub>3-4</sub> or GS  $\geq$  7B. Among the 90 survivors, the response rate to a survey of self-reported HRQoL was 71% (n = 64). The survey occurred 10–16 years after radiotherapy (median, 11 years), and the responders' ages were 63–87 years (median, 78 years) at that time. Thirty-one of the responders (48%) had progressed clinically and/or used HT because of PSA recurrence at the time of the survey. The others were regarded as clinically free of progression when the HRQoL data were analyzed.

#### *Clinical outcomes*

All 203 patients were followed for at least 10 years except for two patients who were lost to follow-up after one and five years because of emigration. The 10-year probabilities of overall survival, cancer-specific survival, and clinical progression-free survival were 57%, 72% and 45% respectively (Fig. 1A, paper I). The overall death rate remained relatively constant for 15 years after therapy, whereas the risk of clinical progression tended to decline after 10 years. Compared with high-risk patients, low-risk patients (cT<sub>1-2</sub> and GS  $\leq$  7A) had significantly better overall survival (79% versus 52% at 10 years), cancer-specific survival (95% versus 66% at 10 years), and clinical progression-free survival (73% versus 39% at ten years) (Fig. 2, paper I). Increasing GS ( $\leq$  7A versus  $\geq$  7B) and cT category (cT<sub>1-2</sub> versus cT<sub>3-4</sub>) were independently associated with decreasing cancer-specific survival and clinical progression-free survival (Table 2, paper I). The GS was the only independent predictor of overall survival. The pretreatment PSA concentration was not associated with any of the endpoints (Fig. 1D, paper I). Compared with age-matched controls from the general population, high-risk patients had significantly poorer overall survival; the 10-year rates were 48% and 74%, respectively (p < 0.00005) (Fig. 3, paper I). Overall survival did not differ significantly between controls and patients with low-risk tumors.

### *Sexual function*

Eighty-seven percent of respondents had not experienced firm enough erections for sexual intercourse during the previous four weeks, and 63% had felt no sexual drive. The mean BSFI scores in patients who had progressed clinically and/or were on HT at the time of response were all lower than in those who were clinically free of progression (Fig. 4A, paper I). Compared with the normative data (171), the mean patient scores were lower within the sexual function domains of the BSFI and in overall sexual satisfaction (Fig. 4A, paper I). The scores for the clinically progression-free patients were similar to the normative data in the sexual problem domain.

### *Urinary and bowel function*

Twenty-three percent of the patients had urinary leakage at least every week and 16% every day (Table 3, paper I). Twenty-five percent of patients used pads on a daily basis because of urinary leakage. Forty-four percent (n = 27) reported some degree of fecal leakage over the previous four weeks. Twenty of these (74%) had had involuntary leakage of feces less than once a week, seven about once a week, and none every day. Fifteen percent of all patients used pads because of fecal leakage on a daily basis.

Nineteen percent of responders without clinical progression or HT and 14% of patients with clinical progression or HT regarded their urinary function to be a moderate or big problem. In both groups, 19% regarded their bowel habits to be a moderate or big problem.

Compared with older American men from the general population, patients had poorer urinary function, more urinary bother, and more bowel bother but similar bowel function (Fig. 4B, paper I). Compared with Norwegian normative data, patients reported clinically significantly more diarrhea when completing the QLQ-C30 (Table 4, paper I).

### *General HRQoL (QLQ-C30)*

Multivariate analysis showed that better physical function and less pain were independently associated with better global HRQoL in the responders. Organ-specific outcomes, including the sexual function domains, did not independently impact on global HRQoL.

Fifty-five of the 64 responders had been included in a similar cross-sectional survey about two years after radiotherapy (172). At that time, 54 had no evidence of clinical progression. In this subpopulation, the answers from both surveys were compared with each other in a longitudinal fashion. In the second survey, the mean outcomes were worse for most domains (Fig. 5A, paper I). The differences between mean scores tended to be greater in

patients who had progressed clinically and/or were on HT at the time of the second survey compared with progression-free patients. These findings corresponded to larger reduction in global HRQoL and function domains and more aggravation of symptoms in those who had progressed and/or used HT compared with those who remained free of progression (Fig. 5B, paper I).

Compared with Norwegian normative data, patients had similar scores on global HRQoL (Table 4, paper I). The patient-scores for the QLQ-C30 domains of physical, role, emotional, and cognitive function were also scored similar to the normative data. Social function was also similar in the general population and patients without clinical progression, but patients who had progressed clinically and/or used HT had clinically poorer social function (difference in mean score > 10). The scores for the nine symptom domains of the QLQ-C30 were similar in patients and normative data except that patients had more diarrheas, and those who had progressed clinically and/or used HT had more insomnia.

### **2.4.2 Paper II**

The aim was to assess self-reported HRQoL in PCa patients who had used a nonsteroidal AA continuously for five years as adjuvant treatment to definitive radiotherapy within the framework of a randomized trial (105) and compare the results to HRQoL among cancer-free, age-matched controls from the general population. Longitudinal changes in serum concentrations of sex hormones and correlations between sex hormone concentrations and HRQoL were also explored.

The SPCG-7 was designed to compare AA monotherapy with AA + definitive radiotherapy for locally advanced or localized aggressive PCa (261). Patients were included from several institutions during 1996–2002. In our side study, only patients who were randomized to AA + radiotherapy at the NRH were included. As part of a scheduled five-year follow-up at the NRH, patients were invited to complete a questionnaire if they still used AA as their only anticancer medication and were judged to be free from distant metastases. The questionnaire differed from that used in the SPCG-7. Eighty-six of the 89 eligible patients responded (response rate of 97%). The median age at the time of the survey was 71 years (range, 56–79 years). Data for age-matched control cohorts were retrieved as described in the methodology section.

### *General HRQoL (SF-36)*

Compared with the controls, patients scored lower on the SF-36 domains of physical functioning, general health, and vitality (Table 3, paper II). In contrast, patients had better outcomes in role limitations due to emotional aspects and mental health. The SF-36 physical component summary score was higher in controls than in patients, whereas the mental component summary score was higher in patients than in controls. Although these differences were statistically significant, they were not clinically significant according to the calculated effect sizes, which were  $< 0.4$  for all domains.

### *Anxiety, depression and fatigue (HADS, FQ)*

HADS caseness of anxiety was found in 17% of patients and in 18% of controls ( $p = 0.84$ ) (Table 3, paper II). Caseness of depression was found in 17% of patients and 11% of controls ( $p = 0.37$ ). Sixteen percent of patients and 12% of controls had chronic fatigue ( $p = 0.38$ ). The domain scores of depression and anxiety, and mental, physical, and total fatigue were similar in patients and controls.

### *Sexual function (BSFI)*

According to the predefined definitions, 64% of the patients were sexually dissatisfied compared with 19% of controls ( $p < 0.001$ ; effect size, 0.95) (Table 3, paper II). Seventy-eight percent of patients and 16% of controls had erectile dysfunction ( $p < 0.001$ ; effect size, 1.34), and 55% of patients and 8% of controls lacked sexual drive ( $p < 0.001$ ; effect size, 1.10). All sexual domain scores were both statistically ( $p < 0.05$ ) and clinically (effect size  $\geq 0.4$ ) lower in patients compared with controls.

### *Hormonal changes*

The concentrations of testosterone, estimated free testosterone, estradiol, SHBG, FSH, and LH all increased significantly during the five years of AA treatment (Table 4 and Fig. 1, paper II). No significant correlations were found between any of the HRQoL domains and the concentrations of estimated free testosterone, estradiol, FSH, or LH at the time of the survey.

### 2.4.3 Paper III

The purpose of this study was to explore whether DTC-status in the BM of patients without overt metastases is associated with clinical or histopathological factors at diagnosis or with clinical outcome subsequent to definitive radiotherapy. During 1994–2004, conclusive BM aspirates were sampled from 266 patients referred to the Department of Oncology, NRH because of newly diagnosed cT<sub>1–4</sub>pN<sub>0</sub>M<sub>0</sub> PCa. The relationship between DTC status and other factors at diagnosis was studied in all patients. The prognostic impact of having DTCs in the BM at diagnosis was studied among patients who had completed definitive radiotherapy and had at least one poor prognostic factor at diagnosis (defined as cT<sub>3–4</sub>, PSA  $\geq$  10  $\mu$ g/L or GS  $\geq$  7B) and who started treatment at least five years before the study's cutoff date (n = 131).

#### *Prevalence of DTCs prior to treatment*

Eighteen percent of all 266 patients (n = 48) had positive DTC status before treatment. The number of DTCs per 2 $\times$ 10<sup>6</sup> mononuclear cells were 11 in one patient, four in two patients, three in six patients, two in nine patients and one in 30 patients. Positive DTC status was significantly associated with increasing GS (p = 0.04, Fig. 1, paper III) and increasing percentage of G4/5 (p = 0.04). DTC status was not associated with cT category, PSA concentration, or dichotomized GS ( $\leq$  7A versus  $\geq$  7B) (Table II, paper III).

#### *Prognostic impact of DTCs in the BM before treatment*

With a median follow-up of seven years, the presence of DTCs at diagnosis tended to predict the development of distant metastases as the first clinical relapse (p = 0.07) but was not associated with any other endpoints of failure (Table III, paper III). The seven-year risks of distant metastases as first clinical relapse were 21% for DTC-positive patients versus 6% for DTC-negative patients (Fig. 2A, paper III). Clinical outcome did not differ between patients with one DTC per 2 $\times$ 10<sup>6</sup> mononuclear cells and those with more than one DTC per 2 $\times$ 10<sup>6</sup> mononuclear cells. Among patients with a GS  $\geq$  7B, positive DTC status was significantly associated with greater risk of distant metastases as the first clinical relapse (p = 0.04); the seven-year probabilities of DTC-positive and DTC-negative patients were 34% and 10%, respectively (Fig. 2B, paper III). DTC status was not associated with the development of distant metastases in patients with GS  $\leq$  7A (Fig. 2B, paper III). Seventy-five of the patients in the follow-up cohort had received radiotherapy as monotherapy. Among these, the risk for distant metastases as the first clinical relapse was also significantly higher for DTC-positive

patients than for DTC-negative patients; the seven-year probabilities were 28% and 9%, respectively ( $p = 0.03$ ) (Fig. 3, paper III).

#### **2.4.4 Paper IV**

The purpose was to explore the prevalence of DTCs in the BM of clinically progression-free PCa patients two or more years after definitive radiotherapy with or without HT. Conclusive BM samples were obtained from 130 clinically progression-free patients following radiotherapy monotherapy (treatment group 1,  $n = 56$ ); radiotherapy + neoadjuvant, concomitant, and adjuvant medical castration for a total of two or three years (treatment group 2,  $n = 27$ ); or radiotherapy + continuous nonsteroidal AA (treatment group 3,  $n = 47$ ). The median time from the start of treatment to BM sampling was five years (range, 2–8 years). All patients in treatment group 2 had been without HT for at least one year before BM aspiration, and all in treatment group 3 still used AA. At the time of BM sampling, 20 patients (15%) had developed PSA recurrence. The longitudinal changes in DTC status were studied in a subgroup of 109 patients who had donated BM before treatment.

##### *Prevalence of DTCs in BM following radiotherapy*

Overall, 22 patients (17%) had DTCs in their post-radiotherapy BM. The numbers of DTCs per  $2 \times 10^6$  mononuclear cells were one in 15 patients, two in four patients, three in one patient, four in one patient and 11 in one patient. The posttreatment DTC status was not associated with pretreatment PSA concentration, cT category, GS, risk stratification, or treatment group (Table II, paper IV). The posttreatment DTC status was not associated with the concentrations of PSA or testosterone at the time of aspiration (Tables III and IV, paper IV).

##### *Longitudinal changes in BM status*

Of the 14 patients who had a positive DTC status before treatment, only one (7%) remained positive in the posttreatment sample. Eighteen (19%) of the 95 patients who were DTC negative before treatment were DTC positive after treatment, and the posttreatment DTC status did not differ between treatment groups (Table V, paper IV).

## **2.5 General discussion**

### **2.5.1 Methodological considerations**

#### *Clinical endpoints in survival analyses (papers I and III)*

The endpoint overall survival was based on reliable data from the Central Population Registry. Death in a patient because of PCa was determined retrospectively based on the available medical records. The same was done for the endpoints clinical progression-free survival in paper I and distant metastases as the first clinical relapse, local failure as first clinical relapse, and biochemical failure in paper III. The accuracy of these endpoints was influenced to some degree by the intensity of diagnostic procedures during follow-up, which was not predefined. Both local failures and distant metastases may have been underreported in patients for whom HT had been prescribed because of increasing PSA without a prior digital rectal examination or imaging. However, we believe that most cases with symptomatic distant metastases were identified. Because the patients were followed by many different clinicians, we do not suspect a systematic underreporting of clinical events. In addition, the endpoints were determined before matching with DTC status in paper III. Nevertheless, we cannot exclude the possibility of biased distributions of underreported clinical events in the different groups. The results in paper III should be reproduced in studies that base the scoring of clinical endpoints on prospective follow-up protocols.

#### *HRQoL assessment (papers I and II)*

Cross-sectional studies of HRQoL following treatment can provide only associations. In particular, strong conclusions about treatment-induced side effects cannot be drawn because baseline data are missing. In addition, self-reported outcomes may be confounded by factors such as age, disease progression, and comorbidity. The optimal study should comprise both baseline data and a randomized untreated control group. No such study of the radical treatment of PCa has been published. Comparison with cross-sectional data from normative samples, as done in papers I and II, is another approach to provide insight into cancer-related morbidity. The validity of such comparison depends on whether each sample is representative for the population of interest. In paper I, the mean patient scores were compared directly with published mean scores from the general Norwegian and American populations. Because of possible cross-cultural differences in self-reported outcomes (262), a substantial risk of unrecognized bias, and small sample size in the patient groups, the validity of such comparison may be questioned. We chose to present the mean scores without performing statistical analysis. In paper II, we retrieved data for age-matched control cohorts from a

sample of cancer-free Norwegian men that were similar to the patients regarding level of education and paired relations, which gave smaller risk of sampling bias than that in paper I. However, this may have occurred because of the low response rate of the controls (~35%). A higher response rate is difficult to achieve in population-based surveys on sexual function (169). The response rate of 97% among patients was very satisfactory. However, the study did not include those who had stopped AA treatment before the five-year follow-up, and this may have resulted in a selection bias, i.e. selection of patients who had not experienced unacceptable side effects from AAs.

#### *Detection of DTCs (papers III and VI)*

The sensitivity of assays for DTC detection depends on a number of methodological factors. In the applied standardized method,  $2 \times 10^6$  mononuclear cells were analyzed for DTCs following enrichment by density centrifugation. Even though the sensitivity increased when a more mononuclear cells from breast cancer patients were examined, the predictive impact of early systemic relapse was reduced (263). The explanation may be that a certain load of DTCs is necessary for the development of distant metastases. An increased sensitivity may therefore not be beneficial from a clinical point of view if it hampers the positive predictive value of a test. Nevertheless, increased sensitivity may also be achieved by immunomagnetic separation techniques (74). Such methods may be feasible for isolating a sufficient number of cells for biological characterization but may reduce the specificity and positive predictive value. The sensitivity may be affected by the amount of peripheral blood contamination (201), whether or not DTCs are trapped in the granulocyte compartment following density centrifugation (264), and the anticytokeratin antibody (201).

The specificity of DTC detection must also be considered. False-positive immunostaining of hematopoietic cells can occur (232), and we performed control staining with an irrelevant antibody in addition to careful morphological evaluation of all stained cells (177). Despite these procedures, cells that were evaluated as DTCs have been found in the BM from 4% of female donors without known epithelial cancers (236). It is unknown whether these cells originate from unknown cancers or represent false-positive-stained benign cells that imitate tumor cells. We did not include a control group of men without PCa. This would be difficult to achieve, given the high frequency of undetected prostate tumors, even in men with PSA concentrations  $< 3.0 \mu\text{g/L}$  (see Table 1.2) (265). Therefore, we do not know the exact false positive-rate for the assay applied on men with PCa, but do not expect that it differs significantly from the 4% in women.



Because the BM studies included patients over a 10 year time-period, the methodological development caused some variation in the BM sample processing. As accounted for in paper III, only one negative control slide was stained for each test before 1999 versus four control slides thereafter (232). We cannot exclude the possibility that some DTC-positive patients would have been excluded because of positive scoring both in the specific test and in the negative control if four control slides had been investigated at the earlier times. Furthermore, the manual screening for immunostained slides is vulnerable to intra- and interobserver variability, which may be reduced by automatic screening (233). All slides in paper III and slides from the pre- radiotherapy samples in paper IV were screened manually and most of the post-radiotherapy slides in paper IV were screened automatically.

We do not suspect that any of the factors discussed led to systematically skewed distributions of BM-positive patients within the groups.

## **2.5.2 Discussion of specific results**

### *Paper I*

It is clear that most of the patients in paper I received a suboptimal treatment according to current standards. First, radiotherapy doses  $< 74\text{Gy}$  would now be considered by most radiotherapists to be insufficient to achieve local control (128), even though the optimal radiotherapy dose for different patients remains to be settled (129). Most of the studied patients would today have received adjuvant HT because of poor prognostic factors such as PSA concentration  $> 10\mu\text{g/L}$ ,  $\text{GS} \geq 7$ , or extracapsular tumor extension (94-98;104). Nevertheless, long-term follow-up studies such as ours with clinically robust endpoints such as cancer-specific survival and overall survival serve as important historical references for evaluating newer regimes. The use of overall and PCa-specific mortality is especially important when studying PCa because many clinical studies use PSA recurrence as a final endpoint because of the long follow-up time needed to observe clinical events. In addition, only a few published series have evaluated definitive radiotherapy in surgically staged  $\text{pN}_0$  patients (228;229;266). Together with the 10-year probabilities of 66% for cancer-specific survival and 39% for clinical progression-free survival in our study, the higher death rate in high-risk patients than in age-matched controls from the general population confirms the need for more effective treatment strategies such as dose escalation and/or adjuvant HT for patients with a  $\text{GS} \geq 7\text{B}$  or  $\text{cT}_{3-4}$ . Patients with  $\text{GS} \leq 7\text{A}$  and  $\text{T}_{1-2}$  had similar overall survival rate as did the controls (Fig. 3, paper I). This finding, together with a 10-year cancer-specific survival of 95%, may support active surveillance of selected low-risk patients (112). On the other

hand, the 10-year probability of clinically progression-free survival was 79% in these patients, demonstrating that some of these tumors are clinically relevant. One should bear in mind that our definition of low risk in paper I does not correspond to contemporary definitions of indolent disease in a screening setting (267) because our patients had all clinically detectable or symptomatic tumors.

In our study, of the three traditional prognostic factors — the GS, cT category, and pretreatment PSA — only the GS and cT category were significant predictors of the clinical endpoints. Our results agree with the patterns of care study from 1989, in which pretreatment PSA concentration was an independent risk factor for PSA recurrence but not for clinical failure or death when the GS was included in multivariate analysis (268).

The patients did not receive radiation of the pelvic lymph nodes because of their histopathologically verified pN<sub>0</sub> status. Only a median of eight lymph nodes were removed even though clinical and anatomical studies indicate that extended dissection to remove at least 20 pelvic lymph nodes is required for optimal staging (22;23;269). A certain degree of understaging is suspected, which may have affected the results. Both limited lymph node dissection and widely used nomograms (21;140) probably underestimate the risk of lymph node involvement (26;56;269). These uncertainties about the correct N classification in PCa patients hamper development of adjuvant treatment regimes based on correct staging similar to what has been achieved in breast cancer over the past decades (270).

The poor sexual function compared with the normative data was expected based on longitudinal studies that showed a gradual decline in sexual function the first five years after radiotherapy (164;271). In addition, 44% were on HT at the time of the survey. It is interesting that the sexual problem scores in patients and the general population are similar despite the large difference in sexual function. It seems that the PCa survivors have a different perception of sexual problems than the controls. As discussed in the paper, this may be explained by a response shift among cancer survivors (174).

Twenty-five percent of the respondents used pads because of urinary leakage on a daily basis. In the study by Korfage et al, urinary function was also assessed by the PCI and only 6% used urinary pads at the five year follow-up (164). This discrepancy may indicate late radiation toxicity that leads to increasing urinary leakage after five years but may also reflect the fact that our patients were older, with more urinary comorbidity. Results from a small longitudinal study by Fransson support the first explanation. Urinary incontinence was similar at the four-year and eight-year follow-ups, but had increased markedly at the 15-year

follow-up without a corresponding increase in urinary incontinence in age-matched controls (157).

In paper I, we questioned the ability of the PCI bowel function scale to measure radiotherapy-induced bowel dysfunction because the patients' scores were similar to those from the normative sample. This may also be explained by a gradual decrease in late-onset bowel symptoms following radiotherapy (146). In contrast to our results, in the longitudinal study by Korfage et al, bowel function was reduced by about 10 points on the 0–100 scale from baseline to six months after radiotherapy and remained at that level on the next measurements (164).

The patients' global HRQoL scores 10–16 years after radiotherapy corresponded well with scores from the general population, indicating that radiotherapy has little direct effect on this domain. Although longitudinal studies have shown some inconsistent results regarding changes in general HRQoL domains in the first year after radiotherapy, they generally indicate that the changes, if any, are relatively small (164;272;273). Our results support others who have pointed out that organ-related morbidity may have relatively little effect on self-reported global HRQoL in PCa survivors (164;274). On the other hand, poor urinary function, urinary bother, sexual function, and sexual bother were independently associated with worse generic HRQoL outcomes assessed by SF-36 two years after diagnosis of PCa in a large American population-based study (173).

The longitudinal reduction in the global HRQoL and general function domains observed in our patients compared with a similar survey two years after radiotherapy was most likely confounded by age (170) and disease progression (Fig. 5, paper I) but was probably not a specific result of late toxicity from radiotherapy.

## *Paper II*

As discussed in the paper, preserved sexual-function has been thought to be an advantage of AAs compared with castration based therapy (102). However, the evidence has been based on short-term studies with substantial risk of under-reporting of sexual dysfunction (275-277). The patients studied in paper II received long-term AA + radiotherapy. The poor sexual function that they reported compared with the general population may have been confounded by radiotoxicity (153). However, a severe reduction in sexual function associated with long-term AA monotherapy has recently been reported (105). A previous study reported a gradual decrease in erectile function the first 2–6 years of flutamide (103). Together with our results, these studies indicate that long-term AA may reduce sexual function in most patients. A

gradual decrease in sexual function remains an advantage compared with the sudden decrease that often occurs during medical castration.

We expected that the patients would score lower than the controls on the HRQoL domains depression, vitality, physical functioning, and physical fatigue because these domains are believed to be partly dependent on androgen-receptor activity. However, the differences between patients and controls in these domains were small and not necessarily clinically important. Except for the possibility of type II errors because of small sample sizes, one reason may be that AAs do not have significantly affect these domains. An alternative explanation is that the patients had better baseline scores compared with the controls because major comorbidities would have excluded them from definitive radiotherapy. If so, we would not have expected the statistically significantly better general health in controls compared with patients.

The increase in sex hormone concentrations during AA treatment is well known (99;231), but earlier reports had shorter follow-up compared with the five years in our study. As noted in the paper, the consequences of increased sex hormone concentrations are not known. Medical castration achieved by estrogens is associated with cardiovascular toxicity (93). Theoretically, this may also apply to patients with increased serum estradiol concentration because of AA treatment. Although the results provide strong evidence of an increase in sex hormone concentration on a group level, this did not occur in all patients, and some patients had lower concentrations at the follow-up compared with baseline. Possible causes for these different responses are poor patient compliance or pharmacogenetic differences between patients.

### *Paper III*

Before this study, Weckermann et al had shown that pretreatment DTCs in the BM detected by ICC predicted PSA-based progression following radical prostatectomy (76). Our study is the first to show that pretreatment DTC status in BM predicts development of distant metastases after radical treatment for PCa. The hypothesis that these cells are precursors of distant metastases has been strengthened by a recent study showing that pretreatment DTC status predicts PSA recurrence following radical prostatectomy with neoadjuvant HT (72). However, the clinical relevance of detecting these cells in PCa patients is far from settled. PSA recurrence is only a surrogate endpoint for clinical relapse. The endpoint of distant metastases in our study was assessed retrospectively. The clinical relevance of detecting

DTCs in the BM of PCa patients before treatment needs to be confirmed in prospective trials with clinically robust endpoints before the findings can be implemented in clinical practice.

The prevalence of pretreatment DTCs was 40% and 45% in the studies by Weckermann et al and Kollermann et al compared with only 18% in our study even though the same number of mononuclear cells was examined ( $2 \times 10^6$ ). Using an enrichment method that included both negative and positive immunomagnetic selection and the epithelial marker BerEp4, Pfitzenmeyer et al found BM epithelial cells in as many as 75% of PCa patients before prostatectomy (74). The variability between studies may be related to several factors such as the use of different antibodies and differences in the morphological evaluation of immunostained cells (177). The low prevalence in our study may reflect the effort to select patients without regional lymph node metastases by surgical staging and central reviewing of all histopathological specimens.

In contrast to the three studies discussed above, we found that the risk of having DTCs in the BM before treatment increased with an increasing GS. In our study, all biopsies were graded by one pathologist to avoid interobserver variation. The high prevalence of DTCs in the other studies suggests that their methods had less specificity than ours. From a biological point of view, a connection between an increasing GS and DTC status could partly explain why the GS is such a strong predictor of the progression of PCa (68). Because the presence of DTCs in breast cancer is associated with histopathological grade (188), the same would be expected in PCa.

#### *Paper IV*

In this study, the similar prevalence of positive DTC status following radiotherapy with or without adjuvant HT indicates that HT does not kill DTCs. This agrees with results showing that pretreatment DTC status is a predictor of clinical progression despite neoadjuvant HT for PCa (72) and adjuvant HT for breast cancer (188). The inconsistent BM findings at follow-up compared with baseline may reflect a true biological phenomenon but may also be related to the relatively low sensitivity of the method applied as mentioned previously.

## ***2.6 Conclusions related to the study aims***

1. Among patients who received definitive radiotherapy  $\leq 70\text{Gy}$  monotherapy at the NRH between 1989 and 1996, the 10-year overall survival, cancer-specific survival, and clinical progression-free survival were 57%, 72%, and 45%, respectively.  $\text{GS} \geq 7\text{B}$  and  $\text{cT}_{3-4}$  were independently associated with decreased cancer-specific survival and clinical progression-free survival whereas a  $\text{GS} \geq 7\text{B}$  was the only independent predictor of decreased overall survival. Patients with  $\text{GS} \geq 7\text{B}$  or  $\text{cT}_{3-4}$  had reduced overall survival compared with age-matched controls from the general population. In contrast, patients with  $\text{GS} \leq 7\text{A}$  and  $\text{cT}_{1-2}$  had a similar overall survival as controls.
2. Between 10 and 16 years after definitive radiotherapy for PCa, 89% of responders had complete erectile dysfunction, 23% had urinary leakage at least once every week, and 11% had fecal leakage at least once a week. Compared with published normative data, the patients had similar global HRQoL. Pelvic-specific morbidities probably related to radiotherapy were not independently associated with global HRQoL.
3. Compared with age-matched controls from the general population, patients who had been treated by definitive radiotherapy + long-term adjuvant nonsteroidal AA reported markedly decreased sexual function combined with limitations of physical functioning, general health, and vitality.
4. Serum concentrations of testosterone, estimated free testosterone, estradiol, SHBG, LH, and FSH increased significantly in patients who had used AA for five years.
5. DTCs were found in the BM of 18% of patients with  $\text{cT}_{1-4}\text{pN}_0\text{M}_0$  PCa before therapy. The prevalence of positive DTC status increased with increasing GS and increasing percentage of Gleason grade 4 or 5.
6. The appearance of DTCs in the BM seems to predict the development of distant metastases after curatively intended radiotherapy for high-grade PCa.
7. The prevalence of a positive DTC status was 17% following definitive radiotherapy for PCa and was independent of whether the patients had received radiotherapy as monotherapy, adjuvant castration-based HT, or adjuvant AA.

## **2.7 Future considerations**

The current thesis and related studies warrant further clinical investigations to address the following:

1. The optimal radiotherapy dose and adjuvant treatment may differ according to cancer-related and patient-related factors. Both should be included in future randomized trials.
2. The search for better prognostic markers must continue so that treatment can be differentiated according to biological properties of the specific tumor. New prognostic markers should be evaluated by clinical survival endpoints in long-term studies, and not only by PSA.
3. Side-effects from long-term AA treatment should be evaluated further. Possible cardiovascular consequences from increased serum estradiol concentration in response to AA treatment should be addressed specifically.
4. HRQoL following contemporary curative treatment strategies for PCa should be assessed continuously in long-term longitudinal studies that include baseline data and representative control groups.
6. If pre-radiotherapy lymph node staging is continued in future clinical practice, the method should be standardized.
7. The clinical implications of detecting DTCs in the BM of PCa patients remain to be evaluated in large, prospective trials. The prognostic impact of detecting DTCs by different methods such as direct ICC, ICC following immunomagnetic separation, and quantitative RT-PCR should be compared. No studies have evaluated the prognostic value of detecting DTCs following radiotherapy combined with HT. Future studies should also focus on methodological standardization and biological characterization of the cells. Ultimately, specific therapeutic targets may be identified on the DTCs that could guide adjuvant treatment in the individual patient.

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